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# Annals

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# Annals of the Missouri Botanical Garden

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## STUDIES IN THE PHYSIOLOGY OF THE FUNGI

### XII. PHYSIOLOGICAL SPECIALIZATION IN RHIZOCTONIA

SOLANI KÜHN

TAKASHI MATSUMOTO<sup>1</sup>

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#### INTRODUCTION

The study of the diseases induced by *Rhizoctonia* has been undertaken by many investigators in different countries since the first report of the occurrence of this organism in 1728, in France. Nevertheless, the occurrence of this fungus on such a diversity of host plants and the possible existence of distinct forms or races within the species suggest that there are many phases of the subject still requiring extensive investigation.

The chief object of the present investigation was to make a comparative study of such strains of *Rhizoctonia Solani* Kühn as could be obtained from different disease types of the same host.

It is a well-known fact that a culturable fungus may exhibit considerable differences in morphological characteristics and in physiological behavior under the influence of changes in the culture media or other environmental conditions. On the other hand, within the species there may exist forms or races which in no sense represent the effects of simple environmental factors. These races show more or less distinctive and constant morphological and physiological characteristics under any particular

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set of conditions, and these characteristics are inherited; and taken as a whole, they differ from those of any other race under similar conditions.

For the determination of the species, forms, or races, therefore, it is necessary to take into consideration all of the factors which have been referred to, and a comparison should be attempted only after careful observation of physiological and morphological characters, accompanied by extensive inoculation experiments. It is necessary to make comparative studies between the original and reisolated strains, the latter being obtained from the plants used in the inoculation experiments.

### LITERATURE REVIEW

An adequate review of the literature concerning the diseases caused by *Rhizoctonia* may be had by recourse to the papers of Duggar ('15) and Peltier ('16). Especially is the early European literature extensively reviewed in the paper first mentioned. Therefore I will permit myself only a brief review of some of the more important papers closely connected with my present investigation.

Since the first description of *Rhizoctonia* by DeCandolle in 1818, many species have been described by different authors. The Tulasne brothers, who gave the most complete mycological account of the genus, classified all the forms then known as a single species, *Rhizoctonia violacea* Tul., reducing all other names to synonyms. Later Kühn described a new species on potato. This species, at least, he clearly distinguished from the above, and he named it *R. Solani* Kühn.

In the United States, since the first report of the disease of alfalfa mentioned by Webber, who considered it as identical with *Rhizoctonia Medicaginis* DC., many papers have been presented, such as those of Pammel ('91), discussing its occurrence on beets, and of Atkinson ('92), reporting a sterile fungus on cotton seedlings, and later work showed its occurrence on a number of other kinds of seedlings. Nevertheless, credit for the comprehensive account of *Rhizoctonia* in America should be given to Duggar ('99). He studied different types of plant diseases due to a common *Rhizoctonia* and showed that the beet fungus and carnation fungus were identical, although the special

affinities of these could not be given with certainty. Subsequently, Duggar and Stewart ('01) added a large number of hosts subject to *Rhizoctonia* attack and gave proof that the organs, or forms of the organism, exhibited morphologically and in culture the characters of the beet rot and damping-off fungus. Later, Duggar ('15), after a most elaborate study of the common *Rhizoctonia* (designated *R. Solani* Kühn), made the following statement: "In the different strains which have been studied, originating from different hosts, certain minor modifications of the general habit of the fungus in culture have been observed. But these have not seemed to be sufficient to be considered of specific importance, except in the case of the form on the rhubarb." Further, he said: "The subject needs further investigation, but in general it is felt that these differences are such as might be due to permanent differences in the pathological strains, on the one hand, or may be regarded as temporary differences due to the recent environment, on the other." Furthermore, in that paper he discussed more extensively the relationship between the violet root felt fungus described by Tulasne as *R. violacea* and the common *Rhizoctonia*, and gave some of the important and easily observed contrasting features as usually found in these forms. He proposed that the first-named fungus should be designated as *R. Crocorum* (Pers.) DC., inasmuch as the more appropriate descriptive name, *R. violacea* Tul., does not, unfortunately, conform to the international rules of nomenclature.

At about the same time Peltier ('16), after a prolonged study of the common *Rhizoctonia* occurring in America, arrived at the conclusion that all strains studied by him could be included under one species, *Rhizoctonia Solani* Kühn, for no marked specialization was noted in any of the strains. His argument is as follows: "From these inoculation experiments with a large number of different types of plants we must conclude that all the strains studied, which were obtained from a wide range of hosts of diverse geographical origin, can attack the same species of plant and produce the same characteristic symptoms. No marked specialization was noted in any of the strains." From the culture experiments he observed that the growth of the strains was very variable, those from the same host often producing a dif-

ferent type of growth even on the same media, and that the differences in various cultural characters which were shown by strains from unlike hosts were no greater than the differences which might be manifested by two different strains isolated from the same host, or by the same strain after being kept for different intervals of time in artificial cultures. He further stated that measurements of mycelial and sclerotial cells of the fungus showed large variations, not only between strains from different hosts but also between different strains from the same host; therefore no standard could be determined upon as a means of distinguishing the different strains. Duggar ('16) concluded that the common seed-bed fungus in Germany and in France was identical with the damping-off fungus which had been frequently studied in the United States by Atkinson. Rosenbaum and Shapovalov ('17) studied *Rhizoctonia* diseases of the potato in Maine and proposed a new strain of *Rhizoctonia Solani* Kühn based on the idea that the new strain might be distinguished from the more common *Rhizoctonia* (1) by the more pronounced lesions produced when inoculated into injured stems or tubers; (2) by the reaction, growth, and character of sclerotia on definite media; and (3) by morphological characters, especially by the measurement of the short sclerotial cells of the mycelium; and lastly by the diameter of germ tubes.

Ramsey ('17), working on the form of potato tuber disease produced by these fungi, noticed two important phases of the injury: In one of these the external appearance somewhat resembles scab and extends a dry core into the flesh of the tuber; in another the shrinkage of tissues forms a pit or canal in the center of the infected area, frequently suggesting wire worm injury. Concerning the form of the causal fungus, however, no adequate description was given.

In the same year Matz ('17) described a new species of *Rhizoctonia* on figs. According to him the sclerotia of this species are quite different from those of the common forms. Therefore he proposed the name *Rhizoctonia microsclerotia* for this species.

Concerning the studies on specialization of forms in the species here discussed, absolutely nothing has been reported, although the literature dealing with the specialization of other fungi is rather extensive; especially has the work been elaborate in

regard to the rusts and powdery mildews. A brief review of the important studies will not be superfluous in this connection.

Magnus might be considered one of the early investigators in this line. He suggested that a particular biologic form might, by constant association with one host, change its physiological capabilities to such an extent as to develop a new race. Eriksson ('94), in his cross-inoculation experiments with *Puccinia graminis*, observed the evidence of biologic specialization and noticed that the form upon one host species was not always identical with the form upon another. Dietel ('99) also noticed that a rust fungus which had been capable of attacking a number of plants acquired by long association with one species of host somewhat weakened capabilities of attacking other forms. Ward ('02), in his study on the relations between host and parasite in the bromes and their brown rust, suggested that each specialized form of *Puccinia* might during the lapse of time actually become a distinct species. Eriksson ('02) further stated, in a subsequent paper, that the trend of specialization might be different in isolated localities. Furthermore, Ward ('03), in his excellent work concerning the occurrence of a "bridging species," indicated that some forms of bromes might act as "bridging species" in enabling the rust to pass indirectly from one group of bromes to another, although direct transfer was impossible.

Salmon ('04) also observed a similar phenomenon in *Erysiphe graminis*. The same author in his later work showed that the virulence of *Erysiphe graminis* might be changed by certain cultural conditions. By injuring leaves and subjecting plants to heat, etc., the author was able to infect forms which seemed normally immune. Reed ('02, '16), in his diverse cross-inoculation experiments with *Erysiphe graminis*, noticed some considerable variation in susceptibility among the species and varieties of *Triticum*, *Hordeum*, *Avena*, and *Secale*, and defined the existence of biologic specialization. Shear and Wood ('13) stated that *Glomerella cingulata* was exceedingly variable in all its characters so far as they had been studied, although the cause of this variability was not clear. Further, they noticed that no constant or definite relation had been established between the environmental conditions and the most important variations observed. They said: "In any case the evidence accumulated

by others as well as by the writers appears sufficient to justify the conclusion that many of the variations observed and reported here are not entirely due to any effect of simple environmental factors." Further, they said: "The work of Jennings ('11) with *Paramecium* and that of Barber ('07), Will ('90), Beijerinck ('97) and Hansen ('00) on yeasts, as well as that of other authors cited by Pringsheim ('10), demonstrate at least one thing, and that is the actual existence of rather distinct races or strains within species. These races possess more or less distinctive and constant morphological or physiological characteristics which are generally inherited by their progeny and are apparently not primarily dependent upon environmental conditions." Referring to the common rust of wheat, Stakman ('14) says: "On the most resistant varieties, such as Khapli, the spores are often small in size and sometimes abortive." From a study of biologic specialization in the genus *Septoria* Beach ('19) observed that certain species are differentiated into biologic forms. According to him, disease characters as manifested by the host and some morphological characters of certain species of *Septoria* vary with the host and with environmental conditions and are therefore unreliable in taxonomy.

Concerning the specialization of rusts, Klebahn ('17) expressed an opinion, which unfortunately I have been unable to see in the original, but from the abstract by Matouschek ('19) the following is suggestive of the position taken: "Die fluktuierenden Variationen und die Mutationen sind ja Veränderungen, die, wenn auch vielleicht von der Aussenwelt beeinflusst, aus dem inneren Wesen des lebenden Protoplasmas hervorzugehen scheinen—und diese spielen bei Entstehung der Formenunterschiede vielleicht eine grössere Rolle als bei der Ausbildung der biologischen Verschiedenheiten."

I should not neglect to mention also such studies as those of Stäger on *Claviceps*, of Diedicke on *Pleospora*, of Gilbert on *Plowrightia*, of Müller on *Rhynisma*, and of Hesler on *Sphaeropsis malorum*, etc.

#### SOURCE OF MATERIALS

All the strains obtained by me were first isolated by using potato agar, as this medium is very suitable for the mycelial

and sclerotial growth of the fungi studied. This medium also affords a convenient means of separating the various forms into the following major groups: (1) the strains which with growth blacken the agar; and (2) the strains which do not blacken agar.

#### THE STRAINS WHICH BLACKEN AGAR

P1, isolated from a badly affected stem of potato received from Prof. W. T. Horne, California, 1917.

P2, a culture obtained from a potato stem collected by me at Berkeley, California, 1917.

P3, origin similar to the preceding.

L1, a culture of this strain obtained from a very badly infected lettuce plant, greenhouse, Missouri Botanical Garden.

E1, a culture obtained from a diseased egg-plant (3 inches high), Berkeley, California.

H, obtained from Dr. S. M. Zeller, by whom it had been isolated from *Habenaria* sp. (and kept in an incubator for about 2 months).

#### NO PRONOUNCED, AND AT MOST SLIGHT, BLACKENING OF AGAR

P4 was isolated from sclerotia found on potato tuber obtained on the market, St. Louis, 1918.

P5, origin similar to the preceding.

P6, isolated from sclerotia on potato tuber obtained in the market, Berkeley, California, 1917.

B1, isolated from a brown lesion on the stem of white navy bean, given by Miss E. H. Smith, California, 1917. The plant was not badly infected, having comparatively healthy roots and bearing four pods.

L2, from stock culture in the laboratory of the Missouri Botanical Garden. This strain was isolated by one of the former graduate students, but the record of its habitat was not at hand.

B2, obtained from the brown lesion of a certain variety of navy beans collected by Dr. Duggar, 1919.

#### AGAR NOT BLACKENED

P7, isolated by me from a potato stem, Berkeley, California. The plant was not seriously affected, but all parts were considerably dried when found in the late part of autumn, 1917. The



bark of the plant, especially near the ground, was much injured and easily peeled from the stem. A net-work of fungous threads was abundantly seen on the inside of the bark.

D, obtained from a dahlia plant in the garden just mentioned. The plant was perfectly dry and the bark of the plant was very easily removed from the stem. To my great regret I lost the strain during the progress of this study, so that in the present paper I am unable to report any extended result with it. But from the preliminary experimental work done by me during my stay at the University of California, it seems safe to say that it is identical with P7.

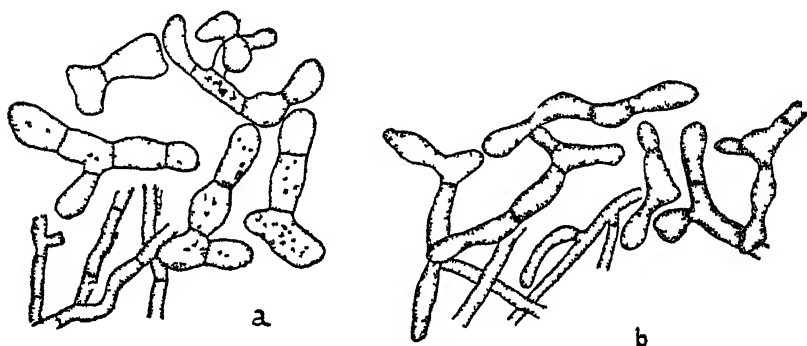


Fig. 1. a, sclerotial and hyphal cells of P1; b, sclerotial and hyphal cells of P4 (camera lucida drawings).

B3, isolated from the *Corticium* stage found on the stems of rather healthy lima beans grown in the Missouri Botanical Garden, 1918. The affected plants appeared practically healthy, having many pods. The *Corticium* stage was also observed on the pods, or leaves, and even on small areas of the soil adjoining the plants.

The account of *Corticium* as a perfect stage of *Rhizoctonia Solani* was first recorded by Rolfs ('03), and subsequently ('04) a more detailed description was published by the same author. The fruiting stage found by him was more or less related to *Corticium vagum* B. & C., but its apparent parasitic mode of life and the size and shape of spores were considered of sufficient importance to establish it as a new variety, and it was designated *Corticium vagum* B. & C. var. *Solani* Burt. However, Burt

('18) later identified the *Corticium* causing disease as the common *Corticium vagum* B. & C., reducing the variety to synonymy. My material was examined by Dr. Burt and determined to be identical with *Corticium vagum* B. & C. For the isolation of the fungus Rolfs suspended the fruiting layer over a Petri dish containing agar, covering stem and dish with a sterile bell jar. If, however, a fruiting stage is too young or too old, this method is unsatisfactory and the dilution method is preferable. The method applied by me consists in touching the hymenial layer with a sterile platinum needle which is immediately removed into sterile water before transfer to melted agar.

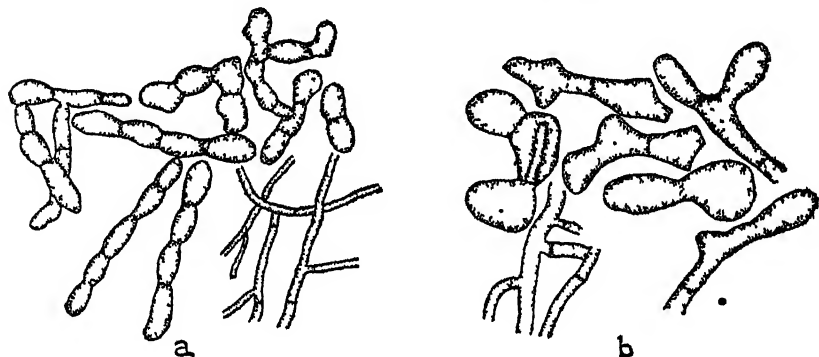


Fig. 2. a, sclerotial and hyphal cells of P7; b, sclerotial and hyphal cells of B1 (camera lucida drawings).

From the preliminary work it appeared that among the strains mentioned above P1, P2, P3, L1, and E1 are very closely related to each other in general morphological characters, in reaction on potato agar, in shape and color of sclerotia, in mycelial characters, etc. Likewise, the group of cultures P4, P5, and P6 are also apparently identical; while another group of similar forms includes B1, L2, and B3.

Therefore, for further extensive studies concerning the physiological specialization of the strains a single representative of each group is considered, namely, P1, P4, P7, B1, B3. In addition to these, H is also included. The last-named strain seemed identical with P1 in every respect, but for the reason that the strain was obtained from a different host and was of different geographical origin, it was decided to use it in the present experiments.

From the cultural experiments with a large number of different media I observed certain marked differences in morphological characteristics of the hyphae and sclerotia exhibited by the 6 strains, most of which are constant, showing no variation for any strain on the same medium and often none on different media. The most striking features shown by the strains will be summarized in the following table:

TABLE I

SHOWING THE MORPHOLOGICAL CHARACTERISTICS OF THE HYPHAE AND SCLEROTIA OF THE 6 STRAINS

|    | Diameter of hyphae ( $\mu$ ) | Size of sclerotial cells<br>Extreme and average measurements ( $\mu$ ) | Color of sclerotia<br>(on corn meal)*                       |
|----|------------------------------|--|---|
| P1 | 8-12                         | 11-17 $\times$ 20-56<br>Average 12 $\times$ 40                         | Brick-red in young, cinnamon-brown or chestnut-brown in old |
| P4 | 8-13                         | 17-23 $\times$ 26-48<br>Average 18 $\times$ 32                         | Chocolate in young, warm blackish brown in old              |
| P7 | 3-6                          | 8-13 $\times$ 15-28<br>Average 9 $\times$ 21                           | Clay-color to tawny olive                                   |
| B1 | 8-14                         | 12-34 $\times$ 29-54<br>Average 27 $\times$ 40                         | Mars brown or chocolate                                     |
| H  | 7-11                         | 8-20 $\times$ 19-48<br>Average 14 $\times$ 38                          | Hazel or brick-red in young, cinnamon-brown in old          |
| B3 | 8-12                         | 14-26 $\times$ 16-42<br>Average 25 $\times$ 36                         | Mars brown or darkish brown                                 |

\* Color designations are in accordance with Ridgway's "Color Standards and Nomenclature."

As shown in the table, the sclerotia of B1 are strikingly large and roundish, while those of P7 are the smallest of all and very light in color. There is also a remarkable difference in diameter of hyphae between P7 and the other strains. In general, there is no striking difference between P1 and P4 and H, though there exist minor differences in color, size, and shape of sclerotia.

The shape of the sclerotia of the various strains will be more clearly demonstrated by the accompanying figures (figs. 1-3).

### INFLUENCE OF TEMPERATURE

Studies on the temperature relations of parasitic fungi are numerous. The experiments and observations alike demonstrate that the growth of a large number of organisms may be closely related to relatively narrow limits of thermal conditions. There are many diseases which develop and spread only during relatively cool seasons, while on the other hand, there are numerous cases which develop only during the hottest weather of summer.

Confining the discussion to parasitic species we shall pass without comment the important work of Wiesner, Tiraboschi, and Thiele. Schneider-Orelli ('12) reported temperature studies on different species or strains of *Gloeosporium fructigenum*, and found that the European form had lower optimum, maximum,

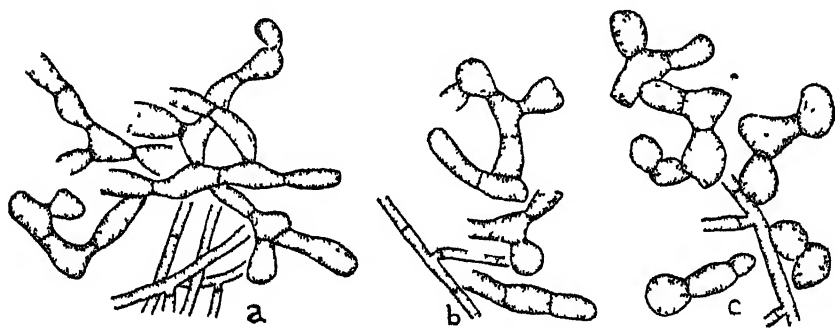


Fig. 3. a, sclerotial and hyphal cells of II; b, sclerotial and hyphal cells of B3; c, sclerotial and hyphal cells of the reisolated strain of B1 (camera lucida drawings).

and minimum temperature than the American form. At 5°C. the European form produced a colony 0.4 cm. in diameter in 12 days and a colony 3.7 cm. in diameter in 35 days, while the American form had made no growth at the end of 35 days.

Brooks and Cooley ('16) noticed that the temperature responses of the various fungi may be greatly modified by the food material upon which they are grown. *Fusarium radicola* and *Glomerella cingulata* had a lower minimum temperature on corn meal agar than on fruit, and the early growth of species of

*Alternaria*, *Botrytis*, and *Penicillium*, etc., was much less inhibited on corn meal agar at low temperature than on the apple. In 1918 Hemmi, working with a large number of species of *Gloeosporium*, obtained from many different host plants, reported a similar relation concerning the maximum temperature of the strain isolated from loquat (*Eriobotrya japonica*). It is clear, therefore, that in any study of the temperature relations of fungi, it is very necessary to take into consideration all environmental factors, and comparative studies should always be performed under the same physiological conditions.

In cultures on potato agar it is observed that there is little difference in the rate of growth of the mycelium of B1 between 27 and 33° C., and the optimum temperature lies within this range, perhaps actually about 31° C. As no growth takes place at 13–15° C., the minimum is relatively higher. Above 33° C. the rate of growth of the same strain gradually decreases, and in most cases, according to my experiments, no new growth is secured at 44°C., showing that this temperature is about the maximum, or perhaps 42–44°C.

Almost the same results are obtained in the germination experiments with the sclerotia of B1. The germination of the sclerotia was studied in distilled water by the hanging-drop method at 34–36° C. to 22–24° C. After a few hours the sclerotia generally began to germinate and at the higher temperature a large number germinated, while at the lower a few only germinated, as shown in table II.

TABLE II

EXTENT OF GROWTH OF B1 AT DIFFERENT TEMPERATURES

| Temperature | After 4 hours | After 8 hours | After 15 hours |
|-------------|---------------|---------------|----------------|
| 34–36° C.   | 24μ           | 89μ           | About 140μ     |
| 22–24° C.   | No growth     | 38μ           | About 95μ      |

In general, the three strains, P1, P4, and H, have about the same rate of growth at the different temperatures, although P4 differs slightly from the other 2 strains. The optimum temperature of these strains would seem to be about 24° C. At 14–15° C. there is still some new mycelial growth, so that the minimum

temperature of the strains is lower than that of B1. With regard to maximum temperature of those 3 strains named above, in most cases no growth is secured at 39–40° C., P4 in every case producing no new growth even at 38–39° C.

Concerning P7, there is no notable difference between 23 and 33° C., and at 14–16° C. there is still more or less growth. The minimum temperature is slightly lower than that of B1. At 37–40° C. the growth is much retarded, the maximum being somewhat lower than that of B1. The germination experiments show that the growth of P7, though slender, is more vigorous in all drop cultures than the remaining strains.

The growth of the mycelium of B3 on culture media is very slow, and at present I find that the strain grows better at a lower temperature (about 22° C.) than at a higher (about 35° C.).

The growth relations of the different strains at certain temperatures may be illustrated by giving in tabular form the results of one of the experiments—in which potato decoction was used as a medium.

TABLE III

GROWTH OF CERTAIN STRAINS AT DIFFERENT TEMPERATURES, PERIOD OF INCUBATION 3 WEEKS, DRY WEIGHT IN GRAMS

| Strain | 38° C. | 22° C. | Room temperature* |
|--------|--------|--------|-------------------|
| P1     | 0.015  | 0.345  | 0.045             |
| P4     | Negl.  | 0.185  | 0.100             |
| P7     | 0.015  | 0.120  | 0.010             |
| B1     | 0.270  | 0.120  | 0.005             |
| H      | 0.020  | 0.300  | 0.050             |

\* This was 14–17° C. at night and 17–20° C. during the day.

Such relations resulting from other experiments are also shown graphically by fig. 4. In general, the growth during the first 2 days is very slight, so that in the figure there is given an average of the observations taken during the first 5 days.

#### NUTRITIVE METABOLISM WITH SPECIAL REFERENCE TO ENZYMATIC ACTIVITIES

In general, the species of fungi exhibit a more or less marked specialization both qualitatively and quantitatively in respect

to secretion of enzymes. Many investigators have also shown that the formation of enzymes is more or less related to environmental factors. Although it has not yet been thoroughly established to what extent environmental factors are efficient in stimulating or retarding the formation of enzymes, brief reviews of some of this work should be presented in this connection.

Katz ('98) studied the regulatory secretion of amylase in *Aspergillus niger*, *Penicillium glaucum*, etc., and found that the effect of various other substances serving together with starch as a source of carbon is in general to inhibit the secretion of

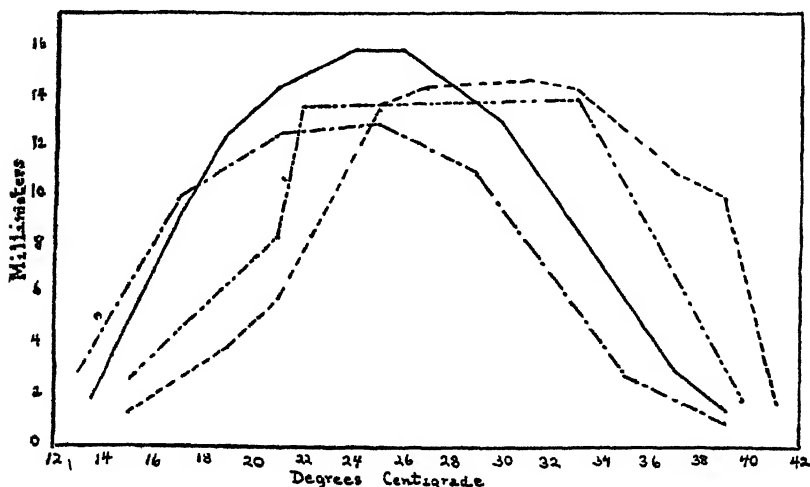


Fig. 4 Rate of growth on bean decoction agar. — represents P1 and P2; — — — P4; - - - - B1; — · — · P7.

amylase, while the presence of starch alone in the culture medium stimulates the fungus to form a large amount of amylase. Herissey ('99) studied the appearance of emulsin in *Aspergillus niger* on Raulin's solution and found that the enzyme was observed only after 48 hours. If 3 to 4 times as much ammonium nitrate as usual were used and this amount replaced at the end of every 24 hours, such cultures grew for a month without sporulation and at the same time yielded no enzyme capable of hydrolyzing amygdalin.

Went ('01), in his study of *Monilia sitophila*, reported that at least 10 enzymes were formed by this fungus and he separated

these enzymes into 3 groups: (1) diastase, etc., which is found at least in small quantity when the fungus is grown on any medium; (2) maltoglucose, formed only when both carbohydrate and a nitrogen-containing salt are present; and (3), those that are formed only when certain substances chemically allied to the substratum are present; thus trehalase is formed when the culture medium contains trehalose.

Dox ('10) believed "that enzymes not normally formed by the organism in demonstrable quantities" could not be developed by special nutrition and that the effect of a particular substratum "is, therefore, not to develop an entirely new enzyme, but to stimulate the production of the corresponding enzyme." Roselli ('11), using *Aspergillus niger*, found that equal amounts of various carbohydrates did not affect the amount of inulase secreted materially, but the amount in the culture medium increased rapidly with age. Kylin ('14) could not find any evidence of qualitative enzyme regulation except in the case of tannase formation by *Aspergillus niger* and *Penicillium* sp., which is conditioned by the presence of tannic or gallic acid in the culture medium. Quantitative regulation, however, was found to be pronounced, and greater in the case of *Penicillium* than in *Aspergillus*.

In 1918 Young studied inulase formation in *Aspergillus niger* and concluded that under all conditions studied inulase was produced by the fungus in appreciable quantities, but in greater amount when inulin (or a related substance) was present in the culture medium. Within the last year Kopeloff and Byall ('20) studied the invertase activity of the spores of *Aspergillus niger* and *Penicillium expansum* and reported that the maximum invertase activity occurred at concentrations of sucrose between 50 and 60 per cent.

Besides those factors mentioned above, it is also possible that many other chemical "stimuli," H-ion concentration, temperature, etc., may influence the formation of enzymes. From such facts one may infer that certain forms of fungi might, by constant association with one host or certain complicated environmental conditions, change their nature in respect to enzymatic activity and ultimately become quite distinct from "other forms" of the same species. To what extent, however,



differentiations caused by such environmental conditions might remain as fixed physiological characters of fungi is an open question.

In the experiments which I have performed I had not in mind being able to distinguish species or races in *Rhizoctonia* by such physiological characteristics as variation in enzymic activity. Nevertheless, the use of carefully arranged experiments of this type with synthetic culture media may afford additional opportunities for the identification of strains.

#### METHOD OF PREPARING ENZYME

The mycelium or felt obtained from a liquid culture was washed several times with distilled water. It was then dried, either by electric fan or heat, and finally transferred to a desiccator. After two days it was accurately weighed and a certain amount of the mycelium crushed and added to a definite volume of distilled water. After being incubated for several hours at 35–40° C., the solution was filtered and a fixed amount of this filtrate employed with the substratum to be studied. The mixed solution was incubated at about 30° C. As an antiseptic 2 per cent toluol was used.

With regard to the proper stage of growth of the mycelium for investigation, Malfitano, in his study of proteolytic enzymes stated that the enzymes showed their greatest activity when the mycelium had reached its maximum growth. Zeller ('16), in his study of *Lenzites saepiaria*, found the greater activity in the mycelium in the case of all enzymes except oxidase, which was greater in the sporophore. Young ('18) also observed that inulase was present in the fungous mycelium in greater amount in the period of sporulation of the fungus and rapidly disappeared after that time.

In my work a qualitative study of the diastase activity in mycelial and sclerotial stages was made. As might be expected, the greatest activity was synchronized with maximum growth. Therefore, in the present investigation it was decided that all the material should be prepared as described as soon as the cultures showed the first sclerotia formation. In general, when the strains are cultivated in potato decoction and kept at 23–25° C., the sclerotia formation of P1 and H was 1 or 2 days

earlier than that of B1 and P4. As a rule P7 produces no sclerotia, so that its mycelium was taken at the time of the collection of material of B1 and P4. Growth of B3 was so slow and poor that sometimes enough material for the investigation was not obtained.

#### CARBOHYDRATE METABOLISM WITH SPECIAL REFERENCE TO CULTURAL AND ENZYME STUDY

Much of the literature dealing with carbohydrases of fungi is discussed in the work of Zeller ('16) and will not be given here. From what has preceded it is apparent that no single temperature adapted for this work would be the optimum for all strains. Nevertheless, 25° C. is favorable for all. Therefore, in the present investigation, if no special mention concerning this factor is given, it will be understood that all the cultures are kept at 24–26° C. After a certain period of incubation the cultures were filtered and the mat of the mycelium washed several times with distilled water and finally dried in the oven after the method described by Duggar and his associates ('17). Each weight was read twice at different times, and an average number was taken for the result. All experiments were made with cultures of the various strains of the same age grown on the same media. The determination of the H-ion concentration, within the limit shown in the following culture media, does not seem to be a limiting factor for the growth of the various forms of *Rhizoctonia*. Therefore in this investigation H-ion concentration may be considered negligible.

#### STARCH AND SUCROSE

*Cultural experiments.*—Of each of the following carbohydrates, glucose, cane sugar, and soluble starch, 2 per cent solutions were prepared, and as solvent the well-known Richards' solution, containing as here modified,  $\text{NH}_4\text{NO}_3$ , 1 gm.,  $\text{KNO}_3$ , .50 gm.,  $\text{KH}_2\text{PO}_4$ , .25 gm.,  $\text{MgSO}_4$ , .25 gm., peptone, 20 gms. and distilled water, 1000 cc.

Twenty-five cc. quantities of each of the above-mentioned solutions were placed in 125-cc. Erlenmeyer flasks. Duplicates of all of these were inoculated with each strain, and the work was done in a culture room in which all dust was thoroughly

precipitated by steam. The result obtained after an incubation of 3 weeks is shown in the following table:

TABLE IV  
DRY WEIGHT OF MYCELIUM OF 8 STRAINS OF RHIZOCTONIA

| Medium<br>contains | P <sub>H</sub> | Strains of Rhizoctonia (gms.) |       |       |       |       |       |
|--------------------|----------------|-------------------------------|-------|-------|-------|-------|-------|
|                    |                | P1                            | P4    | P7    | B1    | H     | B3    |
| Glucose            | 7.0            | 0.140                         | 0.145 | 0.127 | 0.010 | 0.147 | 0.005 |
| Sucrose            | 7.0            | 0.185                         | 0.180 | 0.145 | 0.010 | 0.181 | 0.005 |
| Starch             | 7.0            | 0.165                         | 0.195 | 0.090 | 0.010 | 0.160 | 0.005 |
| Control*           | 7.2            | Negl.                         | Negl. | Negl. | Negl. | Negl. | Negl. |

\* No carbohydrate.

At the beginning the mycelial growth of P4 in the sucrose and starch solutions was rather less in quantity than the glucose solution. Gradually, however, the development became more vigorous, and the final results, as shown above, were better than in the latter. The same was true with P1 and H. This relation held true with P7 in the case of sucrose media, but on the media containing starch this strain showed the poorest growth. Growth of B1 and B3 is very scant on any of the media studied, although B1 can grow fairly well on any of the solid media studied.

The course of development just mentioned makes plausible the supposition that the cane sugar and starch were at first slowly converted by these fungi, but inasmuch as the gradual conversion may after a time keep pace with the requirements (where suitable enzymes are secreted), this may be regarded as a favorable factor. The capacities of these forms to produce the necessary hydrolyzing enzymes became a problem of interest in this work.

*Growth on potato decoction and starch solution.*—Three hundred grams of potatoes were sliced as thin as possible, cooked in 1000 cc. water for 1 hour at about 100° C., then strained through cloth, and 50 cc. of the decoction used in each Erlenmeyer flask. The flasks were autoclaved for 15 minutes at 15 pounds pressure, after which they were inoculated with the various strains.<sup>4</sup> Duplicate flasks inoculated were kept as controls.<sup>4</sup>

After the periods of time mentioned in table v about 5 cc. of the culture solutions for sugar determinations were removed to the transfer room previously mentioned. All the cultures were then again placed in the incubator until the next determination. In this way contamination was entirely avoided and separate flasks were not required for each determination. After

TABLE V  
THE GROWTH OF VARIOUS STRAINS ON POTATO DECOCTION\*

| Strain           | Per. of incub. (days) | Growth   | Reducing sugar (mgs.)† | Starch remaining |
|------------------|-----------------------|----------|------------------------|------------------|
| P1               | 5                     | 2        | 5.82                   | 4                |
| P4               | 5                     | 3        | 9.71                   | 4                |
| P7               | 5                     | 2        | 5.80                   | 4                |
| B1               | 5                     | 1        | 5.82                   | 6                |
| P1               | 6                     | 3        | 5.82                   | 2                |
| P4               | 6                     | 3        | 13.59                  | 2                |
| P7               | 6                     | 3        | 3.88                   | 2                |
| B1               | 6                     | 1        | 10.68                  | 4                |
| P1               | 8                     | .043 gm. | 5.82                   | 2                |
| P4               | 8                     | .043 gm. | 5.82                   | 2                |
| P7               | 8                     | .055 gm. | 3.20                   | 2                |
| B1               | 8                     | .010 gm. | 6.32                   | 4                |
| P4               | 12                    | 2        | 10.14                  | 1                |
| P7               | 12                    | 2        | 3.92                   | 2                |
| B1               | 12                    | 1        | 14.77                  | 2                |
| B3               | 12                    | 1        | 6.32                   | 3                |
| <i>Scl. lib.</i> | 12                    | 1        | 14.77                  | 1                |
| P4               | 15                    | 3        | 16.60                  | 0                |
| P7               | 15                    | 4        | 5.82                   | 1                |
| B1               | 15                    | 1        | —                      | 1                |
| B3               | 15                    | 2        | 20.87                  | 2                |
| <i>Scl. lib.</i> | 15                    | 3        | 21.15                  | 0                |
| P4               | 25                    | .160 gm. | .51                    | 0                |
| P7               | 25                    | .170 gm. | .54                    | 1                |
| B1               | 25                    | .020 gm. | 25.67                  | 1                |
| B3               | 25                    | .100 gm. | 16.45                  | 0                |
| <i>Scl. lib.</i> | 25                    | .175 gm. | .51                    | 0                |

\* Under the columns "Growth" and "Starch remaining" the numerals represent relative amounts, in each case 1 representing a minimum positive quantity.

† Reducing sugar as glucose in 10 cc. of medium.

8 days the experiment was discontinued and dry weights of the mycelial felt were obtained (see table v). The second part of this table represents a repetition of the work, using also *Sclerotinia libertiana* by comparison.

In the next experiments 2 per cent soluble starch was dissolved in the stock solution and 50 cc. of the solution used in the Erlenmeyer flasks as before. In this case, however, 3 flasks were used with each organism.

TABLE VI  
SHOWING THE RESULT OF MYCELIAL GROWTH ON SYNTHESIZED CULTURE MEDIA

| Strain           | Period of exp. (days) | pH  | Growth | Reducing sugar* (mgs.) | Starch remaining |
|------------------|-----------------------|-----|--------|------------------------|------------------|
| P1               | 6                     | 5.8 | 3      | 1.5                    | 2                |
| P4               | 6                     | 6.0 | 2      | 5.4                    | 0                |
| P7               | 6                     | 5.8 | 3      | 1.6                    | 2                |
| B1               | 6                     | 6.4 | 1      | 1.0                    | 3                |
| H                | 6                     | 5.8 | 3      | 1.6                    | 2                |
| B3*              | 6                     | 6.8 | 1      | 0.7                    | 3                |
| <i>Scl. lib.</i> | 6                     | 6.8 | 1      | 0.4                    | 3                |
| Control          | 6                     | 6.8 | 0      | .0                     | 3                |
| P1               | 9                     | 5.6 | 4      | 1.6                    | 1                |
| P4               | 9                     | 5.7 | 3      | 5.3                    | 0                |
| P7               | 9                     | 5.8 | 4      | 1.7                    | 1                |
| B1               | 9                     | 5.9 | 2      | 3.9                    | 2                |
| H                | 9                     | 5.7 | 4      | 1.6                    | 1                |
| B3               | 9                     | 6.7 | 1      | 1.0                    | 1                |
| <i>Scl. lib.</i> | 9                     | 6.5 | 2      | 1.8                    | 1                |
| Control          | 9                     | 6.7 | 0      | .0                     | 0                |

\* Reducing sugar as glucose in 10 cc. of medium.

From the tables it is to be inferred that all the strains used possess diastase, but no quantitative determinations of the amount of sugar produced could be made, since constant utilization of the sugar occurs.

It is also demonstrated by these experiments that all the strains studied have a general tendency to increase the active acidity during growth, and the rate of its increase is somewhat proportional to the amount of mycelium.

*Enzyme study*—For the purpose of estimating the enzymatic activity of these strains, the following experiments were carried out by using mycelial extractions added to the substrata to be studied. For sugar estimation the method described by Shaffer ('14) is employed, with slight modification. Two per cent starch solution was prepared, and to 20 cc. of this solution there was

TABLE VII

A QUANTITATIVE STUDY OF DIASTATIC ACTIVITY IN MYCELIAL AND SCLEROTIAL STAGES

| Stage of growth | Reducing sugar as glucose in 10 cc. substrate (mgs.)* |      |      |      |      |
|-----------------|---|------|------|------|------|
|                 | P1  | P4   | P7   | H    | B3   |
| Mycelium        | 2.54  | 7.44 | 2.14 | 5.95 | 2.23 |
| Sclerotia       | 1.98  | 6.89 | 2.15 | 4.84 | 2.27 |

\* Unless otherwise stated, the amounts of reducing sugar are thus reckoned in all subsequent tables.

TABLE VIII

DIASTATIC ACTIVITY OF THE DIFFERENT STRAINS

| Strain   | Reducing sugar (mgs.) |               | Diastatic activity (rel.) |               |
|----------|-----------------------|---------------|---------------------------|---------------|
|          | After 21 hrs.         | After 48 hrs. | After 21 hrs.             | After 48 hrs. |
| P1       | 7.84                  | ....          | 30.45                     | ....          |
| P4       | 25.75                 | 58.41         | 100.00                    | 100.00        |
| P7       | 5.88                  | 9.80          | 22.83                     | 16.78         |
| B1       | 24.69                 | 41.43         | 95.88                     | 70.93         |
| H        | 5.88                  | 9.80          | 22.83                     | 16.78         |
| B3       | 11.86                 | 14.90         | 46.06                     | 25.51         |
| Control* | Negl.                 | Negl.         | 0                         | 0             |
| P1.      | 12.35                 | 18.95         | 63.50                     | 64.38         |
| P4       | 19.45                 | 29.44         | 100.00                    | 100.00        |
| P7       | 10.78                 | 17.90         | 55.42                     | 60.80         |
| B1       | 13.84                 | 18.95         | 71.11                     | 64.37         |
| Control  | Negl.                 | Negl.         |                           |               |

\* Starch solution

added the same amount of .5 per cent mycelial extraction obtained from mats of the fungi grown on potato decoction. Then the mixture was incubated at 29–30° C. for 4 hours when the sugar determinations were made.

The second part of table VIII, below the horizontal line, represents a repetition of most of the work in the first part of the table. From the experiments it is inferred that the diastatic activity of the strain P4 is notable and beyond that of the remaining strains. B1 is next in order of efficiency. The results obtained with P1 and H are rather similar, and follow B1. Diastatic efficiency of P7 is the least of all. This result is confirmatory of the cultural experiments. No definite conclusion may be drawn for B3 owing to the lack of data, but so far as the present experiment indicates the activity of the strain is rather striking and stands above that of P1, H, and P7. —

Furthermore, this study was repeated several times with the use of extractions obtained from different cultures or cultures of different ages. In all cases, with the exception of a minor modification, those relationships mentioned above have proved rather constant, and no marked variation was noticed in any of the results. These results were also verified by using the alcohol precipitate of mycelium extractions.

A comparative study of the enzyme from the original as contrasted with the reisolated strains was also made. P1 and P4 were isolated from sclerotia on potato tubers obtained from experimentally infected plants (see inoculation experiment No. 5). The original strain P1 was used for comparison. The result obtained after 5 hours is shown below:

TABLE IX  
DIASTATIC ACTIVITY OF ORIGINAL AND REISOLATED STRAINS

|                       | P1   | Reisol. P1 | Reisol. P4 |
|-----------------------|------|------------|------------|
| Reducing sugar (mgs.) | 4.78 | 14.13      | 15.38      |

It appears that the diastatic activity of the reisolated strains of P1 and P4 is 3 times as great as that of the original P1. From

this experiment it will be safe to assume that the diastatic efficiency manifested by original strains may be regarded as a permanent and fixed characteristic, but the enzymatic activity may be modified by a transfer to the host plants, while after continual culture in the laboratory it was rather constant on uniform culture media.

With respect to the presence of invertase in these fungi, the following experiments were performed in the same way as described above but using 2 per cent cane sugar instead of starch.

TABLE X

INVERTASE ACTIVITY OF THE STRAINS AFTER AN INCUBATION PERIOD OF 21 HOURS AND 2 DAYS

|                       | P1    | P4     | P7    | B1     | H     | B3    | Control | Per. (hrs.) |
|-----------------------|-------|--------|-------|--------|-------|-------|---------|-------------|
| Reducing sugar (mgs.) | 5.39  | 18.45  | 14.40 | 12.80  | 4.91  | 5.88  | Negl.   | 21          |
| Invertase activity    | 29.91 | 100.00 | 78.05 | 69.38  | 26.61 | 31.87 | 0       | 21          |
| Reducing sugar (mgs.) | 7.35  | 85.38  | 38.45 | 91.90  | 7.35  | 7.35  | Negl.   | 48          |
| Invertase activity    | 8.00  | 92.90  | 41.84 | 100.00 | 8.00  | 8.00  | 0       | 48          |

This study was repeated several times, using several mycelial extracts obtained from various culture media. With the exception of a slight modification, all the experiments show the same tendency as appears above. All strains possess the power of producing hexoses from cane sugar in excess of the food requirements. Invertase activity of P4, P7, and B1 is relatively high, while that of P1, H, and B3 is poor.

A comparative study was also made of the invertase activity of the reisolated strains of P1 and B1, with the result that these gave respectively 4.38 and 6.11 mgs. reducing sugar as compared with 4.12 mgs. in the original P1.

*Maltose and Lactose.*—To the stock culture solution previously described 2 per cent maltose and lactose were added, 40 cc. of Sach's solution then placed in Erlenmeyer flasks and inoculated. Table XI gives the results after 3 weeks.

As shown by the table, the nutritive value of maltose is about the same as that of dextrose, while that of lactose seems to be much less than that of the other sugars.



For quantitative estimation of maltose activity a 1 per cent, solution of maltose was used as a substrate. Twenty cc. of this solution were placed in each of 13 test-tubes, 2 tubes for each organism and 1 for the control. Five cc. of the extractions (0.5 per cent) of the mycelium of the 6 strains were added to each tube except the control which received 5 cc. of distilled water. All the tubes were placed in an incubator (Ca. 28° C.).

TABLE XI

GROWTH OF THE 6 STRAINS ON MALTOSE AND LACTOSE SOLUTION. THE QUANTITIES REPRESENT DRY WEIGHT OF MYCELIUM IN GRAMS

|          | P <sub>K</sub> | P1    | P4    | P7    | B1    | H     | B3    |
|----------|----------------|-------|-------|-------|-------|-------|-------|
| Maltose  | 6.8            | 0.035 | 0.285 | 0.237 | Negl. | 0.330 | Negl. |
| Lactose  | ?              | 0.140 | 0.035 | 0.065 | Negl. | ..... | Negl. |
| Dextrose | 7.0            | 0.332 | 0.025 | 0.210 | Negl. | 0.307 | Negl. |

TABLE XII

MALTASE ACTIVITY OF THE STRAINS

| STRAINS | Reducing sugar as glucose in 10 cc. substrate (mgs.) |               |
|---------|--|---------------|
|         | After 6 days   | After 12 days |
| P1      | 52.68  | 71.45         |
| P4      | 55.44  | 72.48         |
| P7      | 49.58  | 70.10         |
| B1      | 58.48  | 79.22         |
| H       | 52.95  | 72.15         |
| B3      | 52.68  | 72.15         |
| Control | 47.12  | 48.11         |

No doubt exists concerning the presence of maltase in all these forms of the fungus, but as shown by the table, no marked specialization is noticed in any of the strains. In this connection it is rather interesting to note that the maltose secretion by the fungi in question bears a close relation to the nutrient solution in which the fungi are grown. In all the strains maltase is produced in greater amounts when maltose is used in the culture media. It seems to me, however, that there is no "qualitative" relation of maltase secretion in these fungi, since under

any condition studied maltase is formed at least in small quantities.

In dealing with lactase the same procedure was carried out as for maltase, except that lactose was used as a substrate. Three experiments with the use of mycelial extractions obtained from mats of the fungi grown on potato decoction were made at different periods, but there is no distinct indication of the presence of the enzyme in an extract from any strain. Nevertheless, the secretion of a lactase by these fungi is suspected because of the constant utilization of lactose when the strains are cultured on that medium. In the hope of securing more definite results in this direction further experiments were made with the 6 strains as shown in table XIII.

TABLE XIII

GROWTH OF THE 6 STRAINS ON MALTOSE AND LACTOSE MEDIA AFTER 4 WEEKS, DRY WEIGHT IN GRAMS

| Media   | P1    | P4    | P7    | B1    | H     | B3    |
|---------|-------|-------|-------|-------|-------|-------|
| Maltose | 0.870 | 0.850 | 0.670 | 0.870 | 0.790 | Negl. |
| Lactose | 0.400 | 0.280 | 0.640 | 0.710 | 0.317 | Negl. |

From this experiment it appeared that all the strains were able to utilize lactose as a source of carbon, although its availability was much less than that of maltose.

In dealing with lactose the same procedure as described above for maltase was carried out, except that the extracts were added to 2 per cent lactose solutions and incubated for 1 month. Four determinations were made. The results were rather variable, the quantity of  $K_2MNO_4$  required for 2 cc. of the solution varying from 3.2 to 4.1 cc., the experiments indicating that all the strains may contain a small amount of lactase but of low activity.

*Monosaccharides.*—For these tests 2 per cent glucose, fructose, and galactose dissolved in the stock solution were used, with the conditions as in the previous experiments.

From table XIV it is inferred that all the monosaccharides are directly utilized by the fungi, with approximately equal

availability. No alcoholic fermentation was noticed in any of the strains studied.

*Inulin.*—With inulin as a source of carbohydrate the 6 strains afforded very small yields ranging from .004 gm. dry weight in the lowest to .015 in the highest. Control cultures with maltose as the carbohydrate nutrient were entirely comparable with the results in table XIV; moreover, no inulase could be demonstrated.

TABLE XIV

THE GROWTH OF SEVERAL STRAINS ON HEXOSE-CONTAINING MEDIA.  
THE QUANTITIES REPRESENT DRY WEIGHTS IN GRAMS

| Media     | P1    | P4    | P7    | B1    |
|-----------|-------|-------|-------|-------|
| Glucose   | 0 425 | 0 360 | 0 370 | Negl. |
| Fructose  | 0 445 | 0 220 | 0 352 | Negl. |
| Galactose | 0 315 | 0 247 | 0.300 | Negl. |

TABLE XV

THE GROWTH OF THE STRAINS ON AMYGDALIN AND MALTOSE SOLUTIONS.  
THE QUANTITIES REPRESENT DRY WEIGHT IN GRAMS

| Media     | P1   | P4   | P7    | B1    | II   | B3   |
|-----------|------|------|-------|-------|------|------|
| Amygdalin | .050 | .055 | .060  | .015  | .060 | .... |
| Maltose   | .200 | .235 | ... . | 0.110 | .200 | Poor |

*Amygdalin.*—Emulsin was for the first time discovered in fungi in 1893 by Bourquelot, who found it in *Aspergillus niger*, and by Gerad, who found it in *Penicillium glaucum*. The former ('94) was also able to detect emulsin in many higher fungi found on wood. From a variety of experiments performed by many investigators, it has been made clear that many glucosides, such as amygdalin, arbutin, populin, and salicin are attacked by emulsin secreted by certain fungi yielding, of course, glucose as one of the products of decomposition.

In the present experiments 2 per cent amygdalin was added to the stock solution, likewise 2 per cent maltose was used for comparison, 25-cc. quantities being used in the flasks. The results are for a period of 2 weeks, and in general these indicate a low rate of glucoside consumption.

For the emulsin test 2 per cent amygdalin was used as a substrate. Five cc. of this was placed in a test-tube and 2 cc. of enzyme extraction (1 per cent strength) was added to 1 tube of the amygdalin solution serving as control. After incubating for 1 day at 45° C. the reducing sugars were determined by the Fehling test.

TABLE XVI

QUANTITATIVE DETERMINATION OF THE EMULSIN ACTIVITY OF THE 6 STRAINS. THE QUANTITIES REPRESENT MILLIGRAMS REDUCING SUGAR IN 2 CC. SOLUTION

| P1  | P4  | P7  | B1  | H   | B3   | Control    |
|-----|-----|-----|-----|-----|------|------------|
| 5.4 | 5.6 | 5.6 | 5.2 | 5.5 | .... | Negligible |

In the 5 cases showing positive action the odor of benzaldehyde was easily recognized.

From these experiments it appears that amygdalin is hydrolyzed and then utilized as a source of carbon. It is apparent that while amygdalin was undoubtedly slowly hydrolyzed, its nutritive value is considerably less than that of maltose. Nor was there any marked difference in emulsin activity in the various strains. In connection with this experiment there was obtained for the first time a relatively fair growth of B1 on a maltose medium, while this organism gave no growth heretofore on any of the synthesized culture media. This suggests that the cultural characters of the fungus are by no means invariable, but more or less modified by environment. Subsequent to this experiment the strain B1 was so changed in physiological capacities as to become a very easily culturable form.

*Cellulose*.—Much of the literature dealing with the solution of cellulose by microorganisms was largely discussed in the

work of McBeth and Scales ('13), Zeller ('16), and Schmitz ('19), therefore no literature review of this subject will be given here.

Filter-paper cellulose was prepared in the manner described by McBeth and Scales ('13). Cellulose agar was prepared by adding to the stock solution about 1 per cent of the precipitated cellulose and 2 per cent agar, afterwards sterilizing as usual. Test-tubes were then prepared with about 15 cc. of the solution and after autoclaving all the tubes were cooled without being slanted, then inoculated.

All the fungi grew relatively well on this medium, dissolving large quantities of cellulose. The hydrolysis of cellulose was shown by the fact that the agar became transparent over an area extending considerably beyond the hyphae.

TABLE XVII

GROWTH OF THE STRAINS ON SYNTHESIZED CELLULOSE CULTURE MEDIA  
THE DECIMAL QUANTITIES REPRESENT DRY WEIGHT IN GRAMS

| Strains | Cellulose |       | Maltose |      | No carbon |      |
|---------|-----------|-------|---------|------|-----------|------|
|         | Mycl.     | Scl.* | Mycl.   | Scl. | Mycl.     | Scl. |
| P1      | 0 123     | 1     | 0 255   | 3    | 0 010     | 0    |
| P4      | 0 062     | 1     | 0 252   | 3    | 0 009     | 0    |
| P7      | 0 098     | 0     | 0 275   | 0    | 0 015     | 0    |
| B1      | 0 090     | 2     | 0 245   | 3    | 0 002     | 0    |
| H       | 0 048     | 1     | 0 245   | 3    | 0 012     | 0    |
| B3      | None      | 0     | None    | 0    | None      | 0    |

\*The numerals in this and in subsequent tables represent relative amounts, 1 being the lowest positive amount, in this case being the minimum of positive sclerotial formation.

For a determination of any specialization of the forms in respect to cellulose utilization recourse was had to a liquid culture medium. This medium was also prepared by adding about 1 per cent of the precipitated cellulose to a complete mineral nutrient solution, the cellulose being the only source of carbon. For comparison cultures were made with 1 per cent maltose and also with the salt solution lacking all carbohydrate. All cultures were incubated for 1 month.

Direct determination of cellulase was made by employing the method described by Zeller ('16). Five-cc. quantities of mycelial extraction (0.5 per cent strength) were added to 10 cc. of the precipitated cellulose solution (about 1 per cent by weight), and the result was determined after 1 month.

TABLE XVIII

QUANTITATIVE DETERMINATION OF SUGARS RESULTING FROM THE CELLULASE ACTIVITY OF THE DIFFERENT STRAINS

|                        | Reduction of Fehling's solution |    |    |    |   |
|------------------------|---------------------------------|----|----|----|---|
|                        | P1                              | P4 | P7 | B1 | H |
| A. Enzyme              | 3                               | 2  | 2  | 2  | 3 |
| B. Enzyme (autoclaved) | 1                               | 1  | 1  | 1  | 1 |
| C. Cellulose alone     | 0                               | 0  | 0  | 0  | 0 |

In the light of these results there is no doubt that cellulase is present in the mycelium of the strains studied. Its activity was not measured quantitatively, but the cellulase activity of P1 and H is striking as compared with that of the remaining forms.

The 6 strains were grown in Erlenmeyer culture flasks on a mineral nutrient solution containing sucrose and lactose in amounts ranging from 0.1 to 10 per cent. Using the dry weight of mycelium as a criterion there was, as might be generally expected, a progressive increase in yield in all cases except one, up to 5 per cent, of both the sucrose and lactose series, after a growth period of 1 month. The concentrations from 5 to 10 per cent represented a distinct series growing for a period of 5 weeks, and here too there was in all cases with increasing concentration a progressive increase in growth in the sucrose media, the maximum growth occurring in P1 and B1, 0.020 and 1.100 gms. respectively. In the lactose media growth at the higher concentrations was more or less variable, but the surprising feature of these experiments was the high yields obtained at 5 per cent or more, the maximum being 1.05 gms. in the H strain. In general, the limiting concentrations for growth were not determined.

# NITROGEN METABOLISM WITH SPECIAL REFERENCE TO CULTURAL AND ENZYME STUDIES

Attention was next turned to the problem of the requirements of the strains for nitrogen. Research has long shown that different fungi and bacteria behave in the most diverse manner in respect to a source of nitrogen, and it seemed that an experimental study of the various strains of *Rhizoctonia* might throw some light on physiological differentiation.

TABLE XIX

THE GROWTH OF THE STRAINS ON VARIOUS NITROGENOUS CULTURE MEDIA. THE GROWTH QUANTITIES REPRESENT DRY WEIGHT IN GRAMS

| Solution containing | P <sub>H</sub> | P1        | P4        | P7        | B1        | H         | B3        |
|---------------------|----------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Amygdalin Sclerotia | 6 8            | .012<br>1 | .015<br>1 | .027<br>0 | .007<br>0 | .012<br>1 | .005<br>1 |
| Asparagin Sclerotia | 6 0            | .135<br>3 | .030<br>3 | .070<br>0 | .010<br>0 | .165<br>3 | .010<br>1 |
| Caffeine Sclerotia  | 6 8            | 0<br>0    | 0<br>0    | 0<br>0    | 0<br>0    | 0<br>0    | 0<br>0    |
| Casein Sclerotia    | 6 0            | 155<br>3  | 120<br>2  | .090<br>0 | 520<br>1  | 165<br>3  | 110<br>2  |
| Legumin Sclerotia   | 6.2            | 110<br>1  | .055<br>2 | .100<br>1 | .025<br>1 | 125<br>1  | .020<br>1 |
| Peptone Sclerotia   | 6 8            | .120<br>2 | .120<br>2 | .120<br>0 | .020<br>1 | .160<br>2 | .010<br>1 |

*Cultural experiment.*—For this experiment .5 per cent solutions of amygdalin, asparagin, caffeine, casein, legumin, and bacto-peptone were prepared, each in a medium containing 2 per cent maltose, .025 per cent magnesium sulphate, and .025 per cent monobasic potassium phosphate. Flasks were arranged with 25-cc. quantities and after inoculation were incubated for 16 days.

Concerning the requirements of nitrogen for each strain, there is some specialization exhibited. As a rule the growth of P1 and H in all the media is more abundant than that of the remaining strains, with the exception of B1 on the casein medium.

With respect to the nutritive value of the media used, P1 and H gr̄c̄ v best on casein; peptone, and asparagin, and less on legumin, while P7 grew best on peptone, legumin, casein, and asparagin in the order named. P4 grew best on peptone and casein, showing no apparent difference between the two, and much less on legumin and asparagin. This series of experiments was performed during the earlier part of the investigation. Therefore the strain B1 had not then shown a high capacity for growth on liquid media. The result with this strain on casein was, however, peculiar. B3 was also unable to grow well on any of the media with the exception of casein. As a whole, casein was the most desirable food material for the various strains. Amygdalin, on the other hand, was unsatisfactory as a source of nitrogen, thus bearing out, as far as this experiment may, Nägeli's supposition that nitrogen cannot be assimilated when in direct combination with carbon. Nevertheless, Pfeffer ('99) found that certain fungi were able to grow when supplied with nitrites and might even obtain their nitrogen from amygdalin or potassium cyanide. Caffeine was not utilized by any of the strains.

With respect to the nutritive value of nitrite and nitrate, Pfeffer also states that nitrites are assimilated by nitrite bacteria only, but that they do not serve as a source of nitrogen for mould fungi; yet Winogradsky's observations are not in accord with this view. Many investigations have been made on the comparative value of nitrate and ammonia compounds. It has also been observed that the nitrogen requirements of certain fungi may depend largely upon the source of carbon. For instance, as shown by Fischer, *Bacillus coli* may utilize nitrate in the presence of glucose, but if glycerin is substituted for glucose it does not thrive on nitrate. In the case of *Aspergillus* Czapek showed that the amino acids were preferable to peptone in the presence of glucose.

Although I have not been able to pursue extensively a study of this interesting subject as it relates to *Rhizoctonia*, the following data are interesting as far as they go. In a solution containing 2 per cent maltose, .025 per cent magnesium sulphate, and .025 per cent potassium monophosphate, there were dissolved in different flasks potassium nitrate, potassium nitrite, and potassium cyanide to make concentrations of 5/100 M.



To compare the result with that of organic compounds, 0.5 per cent casein was used in one flask, likewise one flask without a nitrogen-containing compound served as control. Each culture flask, Erlenmeyers of 125 cc. capacity, contained 25 cc. of the medium to be tested, and the incubator interval was 25 days.

TABLE XX

THE GROWTH OF THE STRAINS ON NITROGEN-CONTAINING CULTURE MEDIA. THE DECIMAL QUANTITIES REPRESENT DRY WEIGHT IN GRAMS

| Strain | Potassium nitrite |      | Potassium nitrate |      | Potassium cyanide |      | Casein |      | No. N |      |
|--------|-------------------|------|-------------------|------|-------------------|------|--------|------|-------|------|
|        | Mycl.             | Scl. | Mycl.             | Scl. | Mycl.             | Scl. | Mycl.  | Scl. | Mycl. | Scl. |
| P1     | .040              | 0    | .210              | 2    | 0                 | 0    | .310   | 3    | Negl. | 0    |
| P4     | 0                 | 0    | .050              | 1    | 0                 | 0    | .240   | 1    | Negl. | 0    |
| P7     | 0                 | 0    | .170              | 0    | 0                 | 0    | .180   | 0    | Negl. | 0    |
| B1     | 0                 | 0    | .020              | 0    | 0                 | 0    | .040   | 1    | Negl. | 0    |
| H      | .040              | 0    | .200              | 2    | 0                 | 0    | .330   | 3    | Negl. | 0    |
| B3-    | 0                 | 0    | .020              | 1    | 0                 | 0    | .220   | 2    | Negl. | 0    |

It is interesting that P1 and H are able to utilize nitrogen in the form of potassium nitrite to a certain extent, while the remaining strains can not. The filtrate of the potassium nitrate solution in which P1 and H had grown exhibited a positive color test for nitrite. To what extent, however, this reduction of nitrate and utilization of nitrite may occur with these fungi has not been established. Slight reduction of nitrate was also observed in the potassium nitrate cultures infected with P4 and B1, but absolutely no reduction took place in P7. A solution of 5/100 M of potassium cyanide was rather toxic to all the fungi studied. In general, nitrogen in the form of casein would seem to be most available.

The next experiment was made like the preceding, but with the following inorganic compounds: (1) 5/100 M ammonium nitrate, (2) 5/100 M potassium nitrate, and (3) 5/100 M ammonium nitrate plus 5/100 M potassium nitrate. These compounds were used with 2 per cent glucose in a mineral solution as before.

The highest yield is obtained from potassium nitrate, and the combination of potassium nitrate and ammonium nitrate shows no better result in mycelial and sclerotial growth than the culture which contains either one of these salts. In this experiment P7 shows the highest growth of any of the cultures used.

TABLE XXI

GROWTH OF THE STRAINS ON NITRATES. CULTURE INTERVAL 3 WEEKS  
THE QUANTITIES REPRESENT DRY WEIGHTS IN GRAMS

|  | P1   | P4   | P7   | B1   | H    | B3    |
|--|------|------|------|------|------|-------|
| NH <sub>4</sub> NO <sub>3</sub>                    | .140 | .073 | .160 | .008 | .130 | Negl. |
| KNO <sub>3</sub>                                   | .183 | .... | .207 | .010 | .195 | Negl. |
| NH <sub>4</sub> NO <sub>3</sub> + KNO <sub>3</sub> | .140 | Lost | .136 | .... | .140 | Negl. |

TABLE XXII

GROWTH OF THE STRAINS IN SUGAR BEET DECOCTION AND INORGANIC  
SOURCES OF NITROGEN. THE DECIMAL QUANTITIES REPRESENT  
DRY WEIGHT IN GRAMS

| Strain | Sugar beet decoction plus 1/100 M. |      |                  |      |                   |      | Sugar beet decoction only |      |
|--------|------------------------------------|------|------------------|------|-------------------|------|---------------------------|------|
|        | Ammonium sulphate                  |      | Ammonium nitrate |      | Potassium nitrate |      |                           |      |
|        | Mycl.                              | Scl. | Mycl.            | Scl. | Mycl.             | Scl. | Mycl.                     | Scl. |
| P1     | .242                               | 2    | .172             | 2    | .204              | 3    | .246                      | 2    |
| P4     | .062                               | 1    | .084             | 1    | .127              | 1    | .237                      | 3    |
| P7     | .185                               | 0    | .185             | 0    | .252              | 0    | .116                      | 0    |
| B1     | .225                               | 2    | .233             | 2    | .223              | 3    | .242                      | 2    |
| H      | .248                               | 2    | .198             | 2    | .226              | 3    | .242                      | 2    |
| B3     | .145                               | 1    | .028             | 0    | .060              | 0    | .060                      | 0    |

After conducting a series of experiments in which (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub>NO<sub>3</sub>, and KNO<sub>3</sub> in concentrations of M/100 and M/500 were added to sugar beet decoction and the strains grown for 10 days without any apparent advantage from the addition of

these salts, a second series was arranged with a culture interval of 4 weeks. The culture vessels and the quantities of solution were as in the preceding series.

The decoction alone seems not only to afford sufficient nitrogen, but with the exception of P7 the addition of these compounds results in decreased growth.

*Proteases.*—Experiments were conducted in the usual way using various substrates. As a source of the enzymes mycelial extracts were used in some cases, while in the others the growing fungus was employed.

*Action on gelatin.*—A 10 per cent solution of gelatin was made up in potato decoction, and about 10-cc. quantities of the solution were placed in test-tubes. The tubes were then immediately autoclaved and slanted. After hardening of the gelatin inoculation was made.

TABLE XXIII  
ACTION OF EXTRACTS OF THE STRAINS ON FIBRIN

|                     | Fresh | Fresh    | Fresh                            | Boiled                            |
|---------------------|-------|----------|----------------------------------|-----------------------------------|
| Substance added     | Water | n/10 HCl | 5Na <sub>2</sub> CO <sub>3</sub> | .5Na <sub>2</sub> CO <sub>3</sub> |
| Fungus, all strains | +     | —        | +                                | —                                 |

All the fungi grew fairly well on this medium, showing no apparent difference in each strain, and after two weeks all the cultures showed liquefaction of the gelatin. Positive qualitative results were also obtained by using mycelial extracts of all the strains.

*Action on fibrin.*—The action on this substrate was determined by placing in each tube 5 cc. of the fresh or boiled extract, a piece of fibrin, also 5 cc. of water, acid, or alkali, and then incubating. The behavior of all strains was positive except in the presence of alkali or where the boiled enzyme was used, so that the result may be expressed in a tabulated summary, as in table xxiii.

*Action on albumen.*—Two per cent of egg albumen was added to a modified Richards' mineral nutrient solution containing

also a 2 per cent dextrose, and this was heated for half an hour in an autoclave. Erlenmeyers of 25 cc. capacity were then inoculated with the 6 strains and incubated at 23° C. for 3 weeks, with the following yields of mycelium: P1, 0.54 gm.; P4, .18 gm.; P7, .015 gm.; B1, .80 gm.; H, .056 gm., and B3, a negligible amount.

A marked hydrolysis of the albumen occurred in P1 and H, with the larger growth quantities.

#### GROWTH OF THE SIX STRAINS IN RELATION TO H-ION CONCENTRATION†

The importance of the reaction of the culture media as a physiological factor with microorganisms is well recognized, consequently the literature dealing with this problem is rather extensive. Adequate reviews of the literature dealing with the subject were recently given by Webb ('19), and it is unnecessary to include such reviews in this paper. Concerning the influence of acid and alkali on the growth of these fungi, however, Duggar ('99), in an early paper referring to remedial measures, made the following statement: "The use of an alkali as a preventive might be logically suggested knowing the rapidity with which the fungus grows on acidulated nutrient media." Peltier ('19), however, in his study on carnation stem rot stated: "The results showed that *Rhizoctonia* can grow on medium which is, within reasonable limits, either acid or alkaline in reaction." No definite determination of the effect of hydrogen ion (or hydroxyl ion) concentration upon the rate of the mycelial growth of these fungi has been made.

In my investigation, although rather preliminary in nature, the attempt has been made to determine the critical and optimum H-ion concentration, such as observed by Meacham ('18) in mycelial growth of four wood-destroying fungi, or by Webb ('19) in the germination of the spores of certain fungi; likewise I have endeavored to ascertain whether there is any specialization of strains in this respect.

In my earlier studies during this investigation the reactions of the various natural decoctions were designated by Fuller's scale. That this is unsatisfactory is now well known (Duggar and associates, '17), so that in the later studies the method of hydro-

gen-ion concentration (Clark and Lubs, '17) was employed, in the manner detailed below.

*Experiment 1.*—Potato decoction was prepared as usual, then neutralized by means of NaOH. The desired reaction was obtained by the addition of HCl or NaOH, the procedure being then, as in many previous cases, to employ 25 cc. in flask cultures. After autoclaving, the actual H-ion concentration was determined with the use of a colorimeter as outlined by Duggar and Dodge ('19), subsequently by Duggar ('19).

The results after 3 weeks' incubation at 25–28° C. are given in the following table. The growth quantities presented in the table are invariably an average of 2 cultures.

TABLE XXIV

THE EFFECT OF H-ION CONCENTRATION ON THE GROWTH OF THE STRAINS IN POTATO DECOCTION, THE UPPER SERIES AT ROOM TEMPERATURE AND THE LOWER AT 30° C., DRY WEIGHT IN GRAMS

| Strains        | P <sub>H</sub> 2.6 | P <sub>H</sub> 3.9 | P <sub>H</sub> 6.0 | P <sub>H</sub> 7.0 | P <sub>H</sub> 8.2 | P <sub>H</sub> 8.9 |
|----------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| P <sub>1</sub> | No                 | 0.128              | 0.076              | 0.080              | 0.074              | 0.040              |
| P <sub>4</sub> | No                 | 0.095              | 0.084              | 0.078              | 0.060              | 0.035              |
| P <sub>7</sub> | No                 | 0.070              | 0.070              | 0.080              | 0.050              | 0.030              |
| B <sub>1</sub> | 0.080              | 0.146              | 0.084              | 0.092              | 0.060              | 0.020              |
| H              | No                 | 0.110              | 0.083              | 0.080              | 0.069              | 0.040              |
| B <sub>3</sub> | No                 | Negl.              | Negl.              | Negl.              | Negl.              | Negl.              |

| Strains        | P <sub>H</sub> 2.8 | P <sub>H</sub> 3.8 | P <sub>H</sub> 4.3 | P <sub>H</sub> 5.7 | P <sub>H</sub> 5.9 | P <sub>H</sub> 6.7 |
|----------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| P <sub>1</sub> | 0.100              | 0.088              | 0.080              | 0.070              | 0.060              | 0.055              |
| P <sub>4</sub> | No                 | 0.080              | 0.054              | 0.065              | 0.060              | 0.051              |
| P <sub>7</sub> | No                 | 0.085              | 0.060              | 0.065              | 0.060              | 0.055              |
| B <sub>1</sub> | 0.125              | 0.095              | 0.075              | 0.060              | 0.068              | 0.060              |
| H              | 0.100              | 0.090              | 0.080              | 0.060              | 0.070              | 0.060              |
| B <sub>3</sub> | No gr.             | No gr.             | No gr.             | No gr.             | No gr.             | No gr.             |

Rather extensive observations were made on the series kept at 30° C., as follows: By the second day, the growth of P<sub>1</sub>, B<sub>1</sub>, and H was evident in the cultures of P<sub>H</sub>3.8, 4.3, and 5.7, while after 5 days the same strains showed growth at P<sub>H</sub>5.9 (natural solution) and P<sub>H</sub>6.7.

*Experiment 2.*—Sugar beet decoction was used in the same manner, and the result after a growth period of 3 weeks is shown in table xxv.

All the strains, in both experiments, showed an increased growth on the acid side. In the first experiment maximum growth of all the strains was obtained where the exponent was  $P_H$  3.9, the highest acidity in the cultures used was  $P_H$  2.6, and no growth was obtained at  $P_H$  2.6 except with B; while, on the other hand, as shown in the second experiment, no notable differences in growth occurred between the exponent 3.0 and 7.0, although at  $P_H$  4.4 there is a slight increase in all of the strains.

TABLE XXV

THE EFFECT OF H-ION CONCENTRATION ON THE GROWTH OF THE STRAINS  
IN SUGAR BEET DECOCTION, THE DECIMAL QUANTITIES  
REPRESENT DRY WEIGHT IN GRAMS

| Strains | $P_H$ 3.0 |      | $P_H$ 4.4 |      | $P_H$ 5.5 |      | $P_H$ 7.0 |      | $P_H$ 8.0 |      | $P_H$ 8.6 |      |
|---------|-----------|------|-----------|------|-----------|------|-----------|------|-----------|------|-----------|------|
|         | Gr.       | Scl. | Gr.       | Scl. | Gr.       | Scl. | Gr.       | Scl. | Gr.       | Scl. | Gr.       | Scl. |
| P1      | .200      | 2    | .225      | 3    | .225      | 2    | .220      | 2    | .180      | 2    | .155      | 2    |
| P4      | .185      | 2    | .190      | 4    | .195      | 3    | .190      | 3    | .160      | 3    | .140      | 2    |
| P7      | .200      | 0    | .170      | 0    | .145      | 0    | ....      | ..   | .105      | 0    | .090      | 0    |
| B1      | .210      | 2    | .225      | 3    | .225      | 2    | .220      | 2    | .200      | 2    | .190      | 1    |
| H       | .220      | 2    | .250      | 3    | .245      | 2    | .230      | 2    | .190      | 2    | .160      | 2    |
| B3      | .185      | 2    | .175      | 2    | .180      | 2    | .180      | 2    | ....      | ..   | .060      | 1    |

No explanation of the diversity of results in these two cases can be given, but my suggestion is that the effect of the H-ion concentration may be more or less related to the availability of the food materials of the media, since we know that the sugar beet decoction is much more desirable for these fungi than the potato decoction.

*Experiment 3.*—A modified Richards' solution was prepared which contained 1 gm. ammonium nitrate, 0.5 gm. potassium nitrate, 0.25 gm. potassium monophosphate, 0.25 gm. magnesium sulphate, and 20 gms. dextrose in 1000 cc. of water, and hydrogen-ion concentration was adjusted by adding  $H_3PO_4$  or  $NaOH$ . Growing the strains as before the results after a growth

interval of 4 weeks are shown in table XXVI, from which it appears that on this medium there is a narrow range of reaction for favorable growth. Sclerotial formation occurred only at  $P_H 4.4$ .

Sclerotia formation in B1 occurred at  $P_H 3.8$ , also  $P_H 4.3$ , and much less in the natural solution; but no sclerotia were formed at  $P_H 6.7$ . From  $P_H 2.8$  or  $3.8$  growth declined more or less consistently in all strains, as the H-ion concentration was diminished. The most notable result among these observations, however, is the extensive growth of B1, after 5 days, apparently due to the higher temperature. The incubator chamber was then lowered to about  $24^\circ C.$ , and the third observation was made after an interval of 12 days. Some new growth of B1 was then apparent at  $P_H 2.8$ , in which hydrogen-ion concentration the remaining strains had failed to make growth. At this time

TABLE XXVI

THE EFFECT OF H-ION CONCENTRATION ON THE GROWTH OF P1 IN A SYNTHETIC NUTRIENT MEDIUM

| Reaction       | $P_H 2.0$ | $P_H 3.0$ | $P_H 4.4$ | $P_H 6.8$ | $P_H 8.2$ |
|----------------|-----------|-----------|-----------|-----------|-----------|
| Dry wt. (gms.) | No        | No        | 0.055     | 0.025     | 0.010     |

sclerotia formation of B1 was abundant at  $P_H 4.3$ , and less at  $P_H 3.8$ , and the same was true of P1 and H, although the rate of growth was much lower. The result after an incubation interval of 4 weeks is shown in the table, and further discussion is unnecessary.

The effect of H-ion concentration on the growth of the strains is rather variable, or rather somewhat related to the media on which the fungi grow. It is almost impossible therefore to name a definite optimum; nevertheless, the following tentative conclusions may be drawn: No marked specialization as to favorable H-ion concentration was observed, although B1 had wider range on the acid side; and (2) in general, these fungi grew well on acidulated media, as observed by Duggar ('99), the favorable hydrogen-ion concentration being about  $P_H 3.8$ .

## FUSION OF HYPHAE

Fusion of hyphae is common in this fungus and may be observed in any culture of the strains studied, especially in the young stages. It is generally considered that the fusion of hyphae occurs either between hyphae of the same parent mycelium or between hyphae arising from separate colonies of the same physiological form. But with regard to fusion between the different strains obtained from different plants of the same host species, or from different hosts, practically no attempt has been made by any of the earlier investigators to determine this point. I have obtained some interesting results, so that a brief mention of these and of the methods involved is necessary.

For direct study the ordinary drop-culture method was employed, and as a medium 5 per cent maltose in water was used. Hyphae or sclerotial aggregates of the same strain or of different strains were separately sown at opposite sides of a drop. All the cultures were incubated at 27° C., and careful and continual microscopical observation was necessary, because the hyphae or sclerotia may grow out in a few hours, then branch profusely, and readily mingle with the hyphae of the other colony or strain.

In any case, when two hyphae of the same strain are sown in a drop, fusion of hyphae readily takes place. In general, there is a slight inhibition of growth at the margin of the two colonies. Fusion of hyphae occurs readily when P1 and H are sown in the same drop culture. With P1 and P4 the growth rate is about the same, and fusion may take place, although it is not so frequent as in the case of P1 and H. There is no fusion of hyphae between P7 and any of the remaining strains. When P1 and B1 are sown together and incubated at about 21° C., the growth of P1 is much more vigorous than that of B1 and the growth of the former inhibits the further growth of the latter; while if the cultures are incubated at a higher temperature (about 28–30° C.), the result is opposite. In no case does fusion of hyphae occur. A precisely similar result is obtained when B1 and H are grown together. No fusion of hyphae has been established between B1 and any of the remaining strains. When B3 is sown opposite to one of the remaining strains, the growth of the first-named is always inhibited by the latter, so that up to the present time no definite data can be given.



When P1 is sown with the reisolated H (see inoculation no. 1) the growth of both strains is about the same, and frequently fusion of hyphae takes place. Fusion was also frequent between two reisolated strains of P1 and P4, both of which were originally isolated from potato tuber (see inoculation no. 5). In no case was the fusion of hyphae observed when P7 was sown with one of these reisolated strains.

These experiments establish the fact that fusion may occur either between hyphae of the same strain or between those of certain different strains. It is not to be inferred that this fusion phenomenon is analogous to that occurring in *Mucor* or other species of *Zygomycetes*. In the cases here reported the fusion took place only between the hyphae of strains which might possibly have originated rather recently from the same form or race, although modified by environmental conditions. The process by no means represents sexual union. This view is also confirmed by certain experiments with mixed cultures, in which the method described by Zeller and Schmitz ('19) was employed, with slight modification. Petri dishes containing sugar beet agar were each inoculated with 3 of the strains in such a way as to have all possible combinations of each. P1, B1, and H grew much more rapidly than the remaining strains, frequently covering the latter. When hyphae of the same strain, but representing different isolations as P1 and H, came in contact, there was usually no influence of the one colony on the other; that is, the mycelium of the two colonies intermixed, showing a straight line at the margin of the two colonies, owing to the slight inhibition of growth. When B1 and any of the remaining strains came in contact there was a slight stimulation of sclerotial formation on the side of B1 at the margin of the two colonies, shown by the heaping up of sclerotia, while P1 and H (or certain other strains) had rather a tendency to produce sclerotia at the opposite side of the contact line.

The influence of one strain upon another was also studied by another method. After considerable growth of these strains on agar plates the agar-penetrated layer was cut into squares (about 8 mm. across) at the border of any two colonies in such manner that each square contained approximately the same amount of the two colonies. Freshly poured agar plates were

then inoculated with each one of these squares. In cases where the plates were inoculated with the squares of P1  $\times$  P4, P1  $\times$  P7, H  $\times$  P4, or H  $\times$  P7, the growth of P4 and P7 was always inhibited by the accompanying strain and no further growth was noticed, while, on the other hand, when the squares with P1  $\times$  B1 or H  $\times$  B1 were used, the results were entirely dependent upon the environment. For instance, if the plates were immediately incubated at rather high temperatures, growth of the strain P1 or H was inhibited by the strain B1, and the plates were entirely covered by the latter, while if the cultures were incubated at a rather lower temperature, quite the opposite result was noticed. In no case was there evidence that heterozygosis occurred. Growth of the strain B3 was very slow and always covered by growth of the other strains so that no data may be secured for this form in contact with others.

In spite of the data presented, these results must be regarded as preliminary, and in further work cytological technique should be employed.

#### AERATION

With various fungi experiments have shown that the effect of inadequate aëration is repression of growth or suppression of fruiting stages. In order to determine the relation to aëration in *Rhizoctonia* a series of flasks of different sizes were used. Fifty cc. quantities of sugar beet decoction were pipetted into each flask. Immediately after inoculation with the 6 strains the cotton plug was pushed slightly down into the neck of each of the flasks and then sealed with melted paraffin. The results after one month are as follows:

TABLE XXVII

THE INFLUENCE OF AERATION ON GROWTH AND SCLEROTIAL FORMATION, DRY WEIGHT IN GRAMS

| Strains | Size of flasks |      |         |      |         |      |         |      |                  |      |
|---------|----------------|------|---------|------|---------|------|---------|------|------------------|------|
|         | 1000 cc.       |      | 500 cc. |      | 250 cc. |      | 125 cc. |      | Control, 125 cc. |      |
|         | Growth         | Scl. | Growth  | Scl. | Growth  | Scl. | Growth  | Scl. | Growth           | Scl. |
| P1      | .205           | Rare | .090    | No   | .025    | No   | .020    | No   | 1.065            |      |
| P4      | .145           | Rare | .040    | No   | .020    | No   | .015    | No   | .540             |      |
| P7      | .180           | No   | .085    | No   | .030    | No   | .010    | No   | .820             | No   |
| B1      | Negl.          | No   | Negl.   | No   | Negl.   | No   | Negl.   | No   | Negl.            | No   |
| H       | .225           | Rare | .100    | No   | .025    | No   | .020    | No   | .980             |      |
| B3      | Negl.          | No   | Negl.   | No   | Negl.   | No   | Negl.   | No   | Negl.            | No   |

The result is so clear as to leave little doubt of the suppression of growth and sclerotial formation owing to the sealing of the flasks. At the same time, however, it should be noted that at the close of the experiment the check flasks were always almost dry, so that the humidity conditions as well as the concentration were different from those of the sealed flasks.

### INOCULATION EXPERIMENTS

*Experiment 1 (a). (Inoculation of "navy beans" with various strains of Rhizoctonia).*—A number of bean seedlings, each in a pot of sterile soil, were inoculated with the 6 strains by placing some mycelium (all from potato cultures of the same age) near the plants about one-third below the surface of the soil. Twelve plants were inoculated with each strain. After a week "damping-off" was noticed in the pots inoculated with P1 and H, 9 plants being affected in each case. The strain B1 was also able to infect the host to a certain extent, as 3 seedlings were affected. The pots inoculated with P4, P7, and B3 were healthy after 2 weeks.

(b). *(Inoculation of navy bean plants with P1, B1, and H).*—Young beans 7 inches high were pricked with a sterilized scalpel and inoculated with each of the 3 strains mentioned, 10 plants being used in each lot. All the plants were supported by bamboo sticks so as to grow erect. Many of the plants were affected, and with such old beans discoloration is observed not only on the infected stems but also on the roots, yet in no case were the plants killed. Pods were also affected, and through the sunken areas of these the hyphae penetrated the seeds and produced small sclerotia on the seed-coats. No distinction between these 3 strains was noticed in symptoms nor in cultural characters.

*Experiment 2. (Inoculation of Lima beans with the six strains).*—Young beans, 5 inches high, wounded by a sterilized scalpel, were inoculated with the 6 strains, 10 plants being used for each strain. After a week distinct reddish brown lesions of various sizes were produced just at the wounds of those plants inoculated with P1, B1, and H, while on the stem inoculated with P4 and P7 only very slight discoloration, with a light-colored sunken area, was noticed. In the check plants only brown punctures resulted, without any further development of lesions. In-

general, when the strains attack such old seedlings as were used in this experiment, no pronounced symptoms are observed. In spite of the lesions mentioned most of the plants seemed to be quite healthy. However, such plants have a tendency to break easily in the region of the infected part.

*Experiment 3 (Inoculation of navy beans with the six strains).—*Seeds used for the experiments were soaked in 10 per cent Javelle water for 2 hours and dried at room temperature. The seeds were sown with the mycelium of the 6 strains in sterilized pots. The final observations were made after 3 weeks, and are given below.

TABLE XXVIII  
INOCULATION OF NAVY BEANS

|                    | P1 | P4 | P7 | B1 | H  | B3 | Check |
|--------------------|----|----|----|----|----|----|-------|
| Seeds used         | 20 | 20 | 20 | 20 | 20 | 20 | 20    |
| Seeds germinated   | 2  | 9  | 11 | 8  | 1  | 10 | 10    |
| Number damping off | 2  | 0  | 0  | 1  | 1  | 0  | 0     |

TABLE XXIX  
INOCULATION OF LETTUCE

|                       | P1 | P4 | P7 | B1 | H  | B3 | Check |
|-----------------------|----|----|----|----|----|----|-------|
| Number of plants used | 12 | 11 | 12 | 12 | 12 | 12 | 12    |
| Number damping-off    | 9  | 2  | 0  | 4  | 11 | 0  | 0     |

*Experiment 4 (Inoculation of lettuce with the six strains).—*Young plants, 2 inches high, were inoculated with the 6 strains by placing some mycelium obtained from potato cultures of the same age near the plants about one-third inch below the surface of the ground. For 2 days all the plants were covered with wet newspaper, and the results above are after 10 days.

*Experiment 5 (Inoculation of potato tubers with the six strains).—*The tubers were sterilized with formalin and inoculated with

the various strains. Each tuber was planted in a pot of sterilized soil and placed in the greenhouse. The results are shown in table xxx.

After 4 months all the plants were dug up, and the tubers very carefully examined. Practically all those from the sections P1, P4, and H presented more or less sclerotia on the surfaces showing apparently no difference in color and form. Numerous sclerotia were also observed on the stolons.

TABLE XXX  
INOCULATION OF SEED-POTATOES

|  | P1 | P4 | P7 | B1 | H  | B3 | Check |
|--|----|----|----|----|----|----|-------|
| No. seed-potatoes used.....                  | 10 | 10 | 10 | 10 | 10 | 10 | 10    |
| No. potatoes sprouted.....                   | 8  | 9  | 9  | 10 | 9  | 10 | 10    |
| No. stem lesions.....                        | 1  | 1  | 2  | 0  | 2  | 0  | 0     |
| No. plants, black speck sclerotia.....       | 3  | 3  | 0  | 0  | 2  | 0  | 0     |
| No. plants, stem lesion and black speck..... | 4  | 2  | 0  | 0  | 3  | 0  | 0     |

In pathogenicity as well as symptoms no marked distinction between P1 and H was noted. Concerning the relationship between P1 and P4 as stated in the section dealing with the cultural experiments, these are not considered distinct species, but may be regarded as only somewhat specialized in physiological and morphological characters.

P7 is not virulent, although it may induce some secondary injury when the plants are physiologically weak. No light is thrown on the relationships between P1 and B1, for absolutely no infection occurred in the section B1. In general, none of these fungi kill the host plant directly under the conditions described.

*Experiment 6. (Planting the tubers showing Rhizoctonia sclerotia).—*Twenty tubers showing *Rhizoctonia* sclerotia were planted in pots of sterilized soil. The plants were removed from the pots and examined after about 4 months. Many of the tubers were then covered with sclerotia, but no other symptoms, such as stem lesion, *Rhizoctonia* scab, etc. were noticed. As a matter of fact, the tubers produced in the pots were very

small or somewhat deformed, but probably not caused by the fungus, since those in the check were similar in type.

*Experiment 7. (Inoculation of egg-plants with the six strains).*  
—Sterilized pots with a few egg-plants (1.5 inches high) were inoculated below the surface of the soil, as in many previous cases, with the 6 strains. After 2 weeks the results observed were as follows.

TABLE XXXI  
INOCULATION OF EGG-PLANTS

| Fungus                      | P1 | P4 | P7   | B1 | H  | B3   | Control |
|-----------------------------|----|----|------|----|----|------|---------|
| No. plants inoculated.....  | 20 | 18 | 16   | 18 | 15 | 10   | 5       |
| No. plants damping off..... | 18 | 10 | None | 9  | 10 | None | None    |
| Per cent diseased.....      | 90 | 56 | 0    | 50 | 67 | 0    | 0       |

TABLE XXXII  
INOCULATION WITH ORIGINAL CULTURE MATERIAL OF P1

| Plants inoculated                     | No. plants used | No. plants infected |
|---------------------------------------|-----------------|---------------------|
| Potato (10 days after sprouting)..... | 10              | 5 (stem lesion)     |
| Lettuce (about 2 inches).....         | 20              | 13                  |
| Egg-plant (about 2 inches).....       | 20              | 12                  |
| Navy beans (about 2 inches).....      | 15              | 7                   |
| Lima beans (about 2 inches).....      | 13              | 6                   |

In general, there were notable differences in the pathogenicity of the different strains, and this was rather consistent for each strain. In every case the virulence of the strains of P1 and H was remarkable as compared with that of the remainder. Nevertheless, I have still some doubt whether the tendencies manifested by the different strains are sufficiently distinctive to be considered as the fixed hereditary characteristics of those strains. Studies in that direction might throw some further light on the differentiation of the strains.

In order to throw further light on specialization in respect to pathogenicity as a factor which might or might not be readily

intensified, the experiments below were arranged. A piece of agar with mycelium was placed in the soil at a distance of about 1 inch from a plant to be inoculated. No wound was made.

TABLE XXXIII  
INOCULATION WITH REISOLATED STRAINS OF P1

| Plants inoculated       | Reis. strain from | No. plants | No. infected | Remarks         |
|-------------------------|-------------------|------------|--------------|-----------------|
| Potato (1.5" high)      | Potato            | 10         | 7            | Stem-<br>lesion |
|                         | Lettuce           | 10         | 5            | Stem-<br>lesion |
|                         | Egg-plant         | 10         | 4            | Stem-<br>lesion |
|                         | Navy beans        | 10         | 6            | Stem-<br>lesion |
|                         | Lima beans        | 10         | 4            | Stem-<br>lesion |
| Lettuce (about 2")      | Potato            | 20         | 19           |                 |
|                         | Lettuce           | 10         | 19           |                 |
|                         | Egg-plant         | 10         | 18           |                 |
|                         | Navy beans        | 18         | 14           |                 |
|                         | Lima beans        | 17         | 9            |                 |
| Egg-plant<br>(about 2") | Potato            | 20         | 10           |                 |
|                         | Lettuce           | 20         | 12           |                 |
|                         | Egg-plant         | 20         | 15           |                 |
|                         | Navy beans        | 20         | 13           |                 |
|                         | Lima beans        | 20         | 9            |                 |
| Navy beans              | Potato            | 15         | 6            |                 |
|                         | Lettuce           | 13         | 6            |                 |
|                         | Egg-plant         | 15         | 6            |                 |
|                         | Navy beans        | 15         | 9            |                 |
|                         | Lima beans        | 15         | 8            |                 |

As shown by the table, the pathogenicity of the fungus is more or less modified by changing the host plants on which it lives. The highest pathogenic efficiency is always secured when an inoculation is made on plants belonging to the same species as the host from which the inoculation material originated.

#### PARASITISM OF RHIZOCTONIA, WITH SPECIAL REFERENCE TO PENETRATION OF HYPHAE

Concerning the nature of parasitism in *Rhizoctonia*, Drayton ('15), in his microscopical examination of transverse and longi-

tudinal sections of the diseased potato stem, showed that cells of the cortex, vascular bundles, and pith were all found to be invaded by mycelium, and finally the vessels became stuffed with the fungus so that one might infer a lessening of the transpiration stream, resulting in undersized tubers or curling of the leaves, etc. Lastly, he states: "Naturally, sometimes infection may be slight and no leaf curling will have occurred, but the evidence offered is sufficient proof for the stem parasitism of the fungus."

Following his work a study of the parasitism of the potato *Rhizoctonia* was also undertaken by Güssow ('17). He states that tips of the young rootlets fall victim to the invading mycelium, the short shoots are destroyed, and the final effect is decreased. Moreover, since the growth of the soil tubers is precluded the production of aërial tubers may occur.

Other papers touching upon this aspect of the subject (Duggar and Stewart, '01; Fulton, '08; and Barrus, '10) are either of minor importance or have been reviewed by Drayton ('15).

From the facts referred to, it will be inferred that the forms here discussed are able to penetrate practically all living plant tissue and to cause disease in the host. Concerning the mechanism of the penetration of the tissue by the hyphae, however, practically no work has been done on this organism. Well known is the work of earlier investigators, especially that of DeBary ('86) on *Sclerotinia libertiana*, who used in part the drop-lets exuded from the fungus, and concluded that the breaking down of the cell walls was due to an enzyme secreted by the fungus. Ward ('88), studying the *Botrytis* on lily, confirmed DeBary's view, finding that the fungus excreted relatively large quantities of enzyme and dissolved its way into the cell wall. The same opinion was maintained by Büsgen ('93), who maintained that penetration of wall and cuticle by *Botrytis cinerea* is not by mechanical means alone. Nordhausen ('98) also agreed with DeBary, but he found that under certain conditions oxalic acid might play a role in dissolving the cell wall. Smith ('02), following the work of Nordhausen, confirmed the responsibility of oxalic acid in destroying the cell wall.

Brown ('15), studying *Botrytis*, affirmed the enzyme viewpoint, but at the same time he found that the fungus excreted a toxic



substance, which was not of the nature of oxalic acid nor an oxalate, as Smith and others claimed.

Concerning the penetration of epidermal cells by the fungus last mentioned, Blackman and Welsford ('16) showed very clearly that this fungus bores through the cuticle in a purely mechanical way. In his second paper Brown ('16) is convinced that, "the infecting germ tubes of *Botrytis cinerea* are unable to affect chemically the cuticle of the host, nor do they secrete any toxic substance which can pass through the cuticle and bring about the death of the underlying cells." When the cuticular obstacle has been penetrated in a purely mechanical way, the underlying tissues are entered.

Büsgen ('18) published a further study on *Botrytis cinerea*, and from an abstract of this paper it appears that he found no cuticular lesions; the cell walls of invaded cells were more or less dissolved, nucleus and chlorophyll bodies were mostly intact, and the fungus produced a poison not of the nature of an enzyme.

More recently Hawkins and Harvey ('19), in a physiological study of *Pythium*, conclude that the fungus secretes a toxin which kills the cells of the potato, likewise an enzyme by which the organism breaks down the middle lamellae of the host cell, but mechanical pressure exerted by the fungous hyphae seems to be the most important factor in cell wall penetration.

#### METHODS IN THE STUDY OF HOST PENETRATION

In the studies on penetration, most of the investigators mentioned above made use of sections of different parts of the host plants obtained as nearly sterile as possible and then incubated with the fungus. This method of study, however, did not seem applicable in my investigation, because under such conditions penetration would take place much more easily than under natural conditions, and the degree of the parasitism of the different strains would not be ascertainable. The study was therefore undertaken under conditions as near natural as possible.

The method employed in the present experiment was as follows: Seed disinfection for pure culture work was carried out after the method of Duggar and Davis ('19). About 30 seeds of the garden pea were placed in a small cheese-cloth bag, and these immersed in a covered vessel containing 10 per cent Javelle

water. After a treatment of 4 hours the bag was transferred for a few minutes to a jar containing sterile water and then again rinsed in a second jar. The contents of each bag were carefully emptied into a sterile Petri dish. The seeds were then transferred to Petri dishes containing potato decoction agar. All these treatments were made in a transfer room in which all dust was thoroughly precipitated by steam.

The dishes were then incubated at about 25° C. After germination took place each one of the seeds free from contamination was transferred to a large sterile test-tube (1 inch in diameter, 8 inches in height) containing damp filter-paper at the bottom. The basal part of the tube was then covered with black paper to prevent exposure to sunlight. All the cultures were then placed in a greenhouse, and after a few days, when the plants had grown to 2 inches, inoculation with the different strains was made.

The first appearance of the disease was generally observed after a few days, showing a remarkable discoloration in the part attacked. The first sign of the disease in the plants inoculated with P1 and H usually appeared at least 1 or 2 days earlier ~~than~~ that in the plants inoculated with P4 or B1. In any case, no disease was produced by B3. The strain P7 is of low virulence, and if it may attack the plants the first symptom appears later, usually after 2 or 3 weeks. In general, the fungi attacked the plants both on stems and roots, but a direct attack of leaves was rather infrequent.

From the microscopical investigations it appears that the hyphae may enter the plant directly through the surface and in no case through natural openings (fig. 5a and b). Of course, it should be remembered that the stomatal condition is greatly modified by environment, consequently the evidence obtained from the saturated conditions of the cultures cannot be readily applied to natural conditions.

Attention would then naturally turn to the question whether the penetration of the epidermal cell wall is effected in a purely mechanical way or by some special secretion of the fungous hyphae. To solve this question a few drops of mycelial extraction of P1 were put on the upper surface of a pea leaf which was placed in a sterilized Petri dish. For comparison another leaf

was infected with the mycelium of P1 obtained from a pure culture. All the leaves used in the experiment were previously washed in running water for an hour, and finally rinsed with sterilized distilled water in a culture room. All the dishes were then placed in an incubator.

After 2 days some discoloration appeared in the leaves inoculated with mycelium, but practically no change was noticed in the leaves treated with mycelium extract. Microscopical investigation also clearly demonstrated that the tissues of the leaves infected with the mycelium was more or less invaded by the fungus and disorganized to a certain extent, while in the other practically no change had taken place. So far as present information goes, this fact may be cited in support of the mechanical theory proposed by Blackman and Brown.

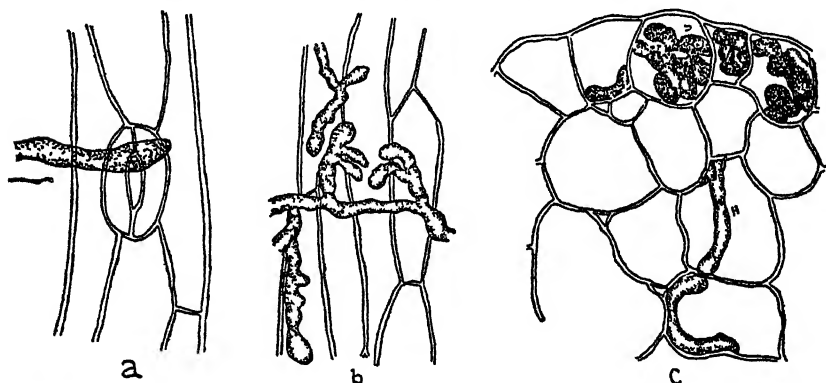


Fig. 5. P1 *a*, hypha not entering through stomata; *b*, swelling of hypha in contact with epidermal cells of pea stem; *c*, penetration of cell wall of pea root by invading hypha (H), young stage of sclerotia formation (S). (Camera lucida drawings.)

If the fungus in question attacks the plant in a purely mechanical way, as discussed above, it would be logical to believe that the infection of the plant by the fungus might take place much easier at the roots than anywhere else, since the epidermis of the roots, unlike that of leaves and stems, has no cutinized walls, especially in the young stage. My attention was, therefore, turned to this point.

For the purpose of this study the following was devised: Young pea plants (more than 4 inches high) grown in pure cultures in test-tubes were pulled out by means of forceps in such a

way as to leave only the roots in the tubes. Then some of these plants were inoculated by placing the mycelium of P1 on the surface of the young roots, after which each plant was supported in the culture tube by means of a sterilized cork at the base of the stem, and the cork was inserted in the tube. To close any air connections between the inside and outside of the tube, the cork was sealed with melted paraffin. The remaining plants were also treated as above, except that the plants were inoculated on the stems instead of on the roots. All these operations were performed in a culture room. The cultures were then removed to a greenhouse and placed under a glass hood, and the atmosphere maintained at the saturation point during the infection period.

Owing to the lack of water in the tubes, the observations were only continued 2 weeks. A few days after inoculation striking brownish discolorations were noticed on the roots inoculated with the fungus, while the plants inoculated on the stems exhibited no positive symptoms. In general, stem infection may take place only in cases where the plants are very young (about 1 or 2 inches high), while the roots are immediately attacked by the fungus. Especially do the young rootlets soon fall victim to the invading mycelium, as observed by Güssow in his study on potato *Rhizoctonia*. Finally, of course, the aerial parts of the plants wilt, because of lack of water supply through the affected roots. As a matter of fact, the loss of water from the tubes containing plants infected through the roots is extremely slight as compared with the others.

Interest was next centered upon the mechanism of penetration of the hyphae after passing through the cuticle. It might be assumed that the penetration of the cell wall is effected by enzymatic action, since these fungi are able to secrete an enzyme which breaks down pure cellulose. Nevertheless, there was still the necessity of determining this point positively. Many sections of roots infected by P1 were made by the method described by Vaughan ('14). Evidence was obtained indicating that the hypha forms a swelling at the end, as it comes in contact with the cell wall, and that it penetrates the wall by a small tube, after which the penetrating hypha usually reassumes its normal diameter. The most interesting feature was that the

hypha usually obtains entrance at a corner where two cells are in contact, in which case the invading hypha frequently grows between the cells dissolving the middle lamella. As shown by some figures (fig. 5c, fig. 6a-d) made at the time of observation, there are also many cases which clearly indicate distortion of host cells following the penetration of the hypha.

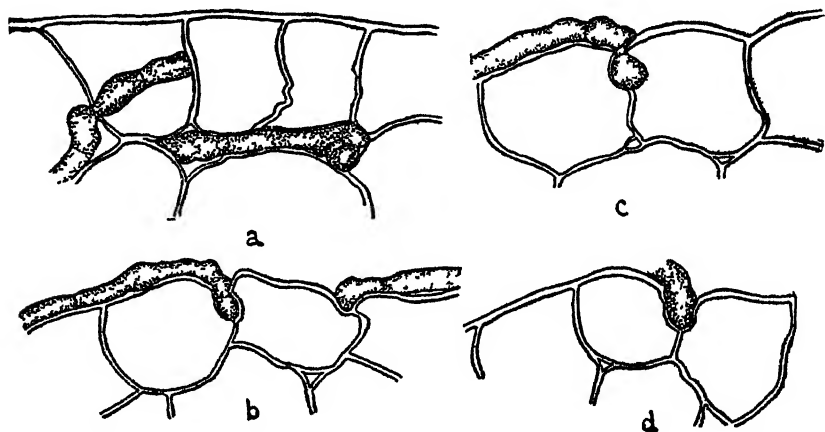


Fig. 6. Camera lucida drawings showing penetration of epidermal cells of pea roots.

From these observations I am of the opinion that the penetration of cell walls may not take place merely in a chemical way, but rather assisted by the mechanical pressure exerted by the fungus. Penetration of the hyphae of P4 and B1 was also noticed to a certain extent, while P7 and B3 seemed to be quite unable to penetrate any living tissue.

#### DISCUSSION OF DATA

From all the evidence at hand it appears that P1 and H are quite identical in their morphological and physiological characteristics, as well as in pathogenicity, and should properly be included under one form of the species *Rhizoctonia Solani* Kühn. This form is a very common type, and the cause of very serious diseases of many cultivated plants. It is very interesting to notice that these two strains, here shown to be identical, are of different geographical origin and from different host plants. I am also inclined to believe that this form of the species may be

more or less closely related to Rosenbaum's new strain, as shown by contrast with his data. Unfortunately no direct comparison could be made with his material.

## ROSENBAUM'S NEW STRAIN

(1) More pathogenic on the stems of potato than other strains or isolations from that host; able to produce a distinct necrosis of the tissues of the potato tuber.

(2) On potato agar this strain produces in 7-10 days a marked discoloration (dark brown to black) of the medium; while other strains, if they produce color at all, never approach the intensity produced by this strain.

(3) On corn-meal agar, there are produced light gray, loosely formed sclerotia, as compared with the darker, brownish, and more compact sclerotia of other strains.

(4) On Uschinsky's solution, after 10 days, the strain covered the surface and was growing on the side of the flask, while in the other growth was still submerged.

(5) The diameter of the hyphae varies from 4.7 to 8.8  $\mu$ , with 7.8  $\mu$  as the average measurement.

(6) Sclerotia: cells measure in length 13.6-30.6  $\mu$ , averaging 21.6  $\mu$ ; in width, 8.3-20.4  $\mu$ , with an average of 12.3  $\mu$ .

## P1 OR H

(1) The strains are more pathogenic on stem of potato, lettuce, egg-plant, and bean than the remaining strains.

(2) On potato agar, these strains produce a blackish discoloration, by which means the strains may be easily distinguished from the others studied.

(3) The characteristics referred to by Rosenbaum are not only observed on corn meal agar, but also on potato agar, bean agar, rice meal agar, and other media.

(4) Unfortunately these strains were not cultured on Uschinsky's solution, but the tendency observed by Rosenbaum is strikingly noticed on all liquid media used.

(5) The diameter of the hyphae of these varies from 7 to 12  $\mu$ , with 9  $\mu$  as the average measurement.

(6) Sclerotia: cells measure in length 17-59  $\mu$ , averaging 38  $\mu$ ; in width, 8-20  $\mu$ .

It is thus shown that with the exception of minor differences in the dimensions of sclerotia the characteristics presented are concordant. Again, it may also be inferred that Rosenbaum's "other strain" may be identical with either B1 or P4.

Concerning the relationship between P1 and P4 there are marked differences in morphological and physiological characteristics, as shown by dimensions and color of sclerotia, by diastase and invertase activities, by temperature requirement, by cultural characters, and by pathogenicity. Nevertheless, these differences may not perhaps be considered sufficient to distinguish these permanently as different species, especially since these characteristics are more or less modified by environment, particularly by a change of host plants. Some important indications obtained by a study of reisolated cultures of these two strains, made from sclerotia appearing on potato tubers artificially inoculated (see inoculation experiment no. 5), are shown below.

## REISOLATED STRAIN P1

(1) Sclerotia: cells 16-23  $\times$  24-48  $\mu$ , dark brown in color.

(2) Hyphae: diameter 8-14  $\mu$ , turning dark brown when old.

(3) Pathogenicity: not so virulent as the original P1.

(4) Cultural characters: blackening of potato agar is not so striking as that of original strain.

(5) Diastatic efficiency: 92 (original strain averages 45).

## REISOLATED STRAIN P4

(1) Sclerotia: cells 16-22  $\times$  23-48  $\mu$ , dark brown in color.

(2) Hyphae: diameter 8-14  $\mu$ , turning dark brown when old.

(3) Pathogenicity: about the same as the original P4 or reisolated P1.

(4) Cultural characters: blackening of agar is about the same as in the original strain P4.

(5) Diastatic efficiency: 100.

The evidence presented above makes it safe to assume that these two strains P1 and P4 may be properly regarded as a single species modified more or less by environment. In general, the strain P4, as Duggar ('16) says, in itself scarcely merits consideration as a causal fungus of disease and I find it less virulent than the strain P1. As a matter of fact, in no case may the strain P4 be so changed as to resemble the form P1, either by changing the culture media or host plants, while P1 may be easily transformed into P4 as has been shown.

The strain B1 is notably distinguished from the remaining strains by its characteristic sclerotia, by its cultural characters, and by having a higher temperature requirement for mycelial

growth, etc. Nevertheless, these characteristics alone are not considered of specific importance on account of indications given later. P1 and B1 were reisolated from affected pods of navy beans used for an inoculation experiment (see inoculation experiment No. 7). Morphological and physiological characteristics of these two reisolated strains may be compared as follows:

| REISOLATED STRAIN P1  | REISOLATED STRAIN B1  |
|---|---|
| (1) Sclerotia: cells 12-19 $\times$ 22-56 $\mu$ .   | (1) Sclerotia: cells 14-21 $\times$ 20-50 $\mu$ .   |
| (2) Hyphae: diameter 8-14 $\mu$ , turning dark brown when old.  | (2) Hyphae: diameter 8-15 $\mu$ , turning dark brown when old.  |
| (3) Invertase activity: (similar to reisolated B1).   | (3) Invertase activity: (similar to reisolated P1).   |
| (4) Temperature requirement: optimum temperature is about the same as that of the original strain P1. | (4) Temperature requirement: optimum temperature is somewhat lower, as compared with that of the original strain B1 (28° C.). |

The characteristics and behavior of these two reisolated strains are rather similar to those of the original strain P1, although the pathogenicity of the reisolated strain B1 is not so pronounced as that of P1. The strains compared may be regarded as two physiological forms of *Rhizoctonia Solani* Kühn.

P7 is strikingly distinguished from the remaining strains by its morphological and physiological characters, and is considered to be new to science. This strain, however, does not seem to be particularly virulent. So far as my present inoculation experiments are concerned, this strain may be responsible for some secondary infections. No definite conclusion may be drawn at present concerning the taxonomic relations of B3, owing to lack of sufficient data.

#### SUMMARY

1. From the macroscopical and microscopical investigation of the 15 different isolations of *Rhizoctonia* obtained from a wide range of hosts of different geographical origin, it was possible at the outset to identify some conclusively, so that the number was reduced to 6 different types for further physiological studies, namely, P1, P4, P7, B1, H, and B3.



2. The temperature requirements of P1 and H are similar, while the remaining strains exhibit different optima, minima, and maxima.

3. All the strains hydrolyze starch, but the diastatic activity is unlike. The activity of P4 is notable and stands above that of the others. B1 has the next higher capacity, and P1 and P7 the minimum.

4. All these fungi are able to convert cane sugar. This inverting activity of P4, P7, and B1 is striking and many times that of P1.

5. Maltase and lactase activity was qualitatively and quantitatively determined, but insufficient data have been collected to determine the extent of specialization. The nutritive value of lactose is markedly less than that of maltose.

6. All the fungi are unable to utilize inulin.

7. Glucose, fructose, and galactose are utilized by these fungi. The availability of these sugars is about the same, and no marked specialization was noticed in any of the strains.

8. Amygdalin is utilized as a source of carbon, being, of course, first decomposed by emulsin before becoming available. No marked difference in emulsin activity was observed in any of the strains studied.

9. Cellulase is present in the mycelium of these strains. Its activity was not measured quantitatively, but qualitative studies indicate that it is highest in P1 and H.

10. P1 and H grow best on casein, peptone, and asparagin as sources of nitrogen and carbon, and less well on legumin, while P7 grows best on peptone, legumin, casein, and asparagin, in the order named. P4 grows best, and equally well on peptone and casein, and it grows less on legumin and asparagin.

11. Potassium nitrate, ammonium sulphate, and ammonium nitrate are available as sources of nitrogen, though potassium nitrate is preferable.

12. P1 and H utilize potassium nitrite, while the remaining strains cannot do so. Reduction of nitrate is observed in the potassium nitrate culture by P1 and H, also P4 and B1. Absolutely no reduction takes place in P7.

13. As a whole, the mycelial growth is more sensitive to modification in the carbohydrate supply than to changes in the nitrogen supply.

14. The growth relations of B1 on liquid media were changed after successive transfers on artificial culture media.

15. No definite cultural characterization of B3 is possible from the results thus far obtained.

16. The presence of trypsin and erepsin was observed in the mycelium of all the strains studied.

17. Examined as to hydrogen-ion concentration all strains show increased growth on the acid side of neutrality. In general, all yield well on media with an exponent somewhat larger than  $P_H$  3.8. P4 and P7 exhibit a somewhat narrower range on the acid side, while B1 has the widest acid range.

18. In these fungi the general tendency is to increase the active acidity during growth, and this increase seems to be proportional to the increase of growth.

19. Fusion occurs between hyphae arising from the different mycelia of the same strain. Fusion is also observed when P1 and H are sown in a drop culture, also between P1 and P4, although this is not so frequent as in the case of P1 and H. No fusion occurs between B1 or P7 and any one of the others. From the experiments it is inferred that fusion may take place only between the hyphae from strains which are rather closely related, or which have rather recently originated from the same ancestral type.

20. The effect of inadequate aëration is to repress the growth of mycelium and sclerotial formation in all strains.

21. From the inoculation experiments it is concluded that P1 and H may attack all the plants studied, generally causing "damping off." B1 also attacks certain hosts, but it is less virulent than the others mentioned. The virulence of P4 is slight. In no case has direct infection by P7 and B3 been observed.

22. The pathogenicity of the strain P1 was more or less modified by a transfer to a host plant different from that on which it was originally found, but the highest pathogenic capacity of the strain is always manifest when inoculation is made on plants belonging to the same species of host as that from which the culture originated.

23. The hyphae of these fungi may enter the host tissue directly through the cuticle, and the penetration of such hyphae is chiefly a mechanical process.

24. Infection of the host takes place much more easily through the root than through any other part.

25. P1 and H are identical in morphological and physiological characteristics and constitute a single form of *Rhizoctonia Solani* Kühn. This form is a very common type of the species, causing serious diseases of many cultivated plants. It may be identical with Rosenbaum's "new" strain.

26. While P1 and P7 might have been derived from the same ancestral origin, some striking physiological specializations have been developed.

27. It is desirable to regard B1 as a physiological strain of *Rhizoctonia Solani* Kühn rather than as a distinct species.

28. P4 and B1 may be two distinctly specialized forms of *R. Solani* Kühn but cannot be considered distinct species.

29. P7 is distinct from all other strains studied, and the differences manifested by this strain may be sufficient to be considered of specific rank.

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## STUDIES IN THE PHYSIOLOGY OF THE FUNGI

### XIII. THE EFFECT OF HYDROGEN-ION CONCENTRATION UPON THE ACCUMULATION AND ACTIVATION OF AMYLASE PRODUCED BY CERTAIN FUNGI

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#### INTRODUCTION

The effect of the hydrogen- and hydroxyl-ion concentration upon the growth of organisms, in general, has received considerable attention in late years, but the effect of these ions upon the individual processes produced by an organism has not been so thoroughly studied. It has been repeatedly shown that certain fungi require an acid medium to produce maximum growth, while others require alkaline conditions. These diverse relations would seem to indicate a difference in the production, accumulation, and activation of the various enzymes concerned in the growth of these organisms. Recent work has indicated a difference in animal and plant proteolytic enzymes, and it has been shown that plant amylases require a range in conditions different from pancreatic amylase, with regard to activity. In plants, secretion amylase has been found to have properties quite different from those of translocation amylase.

It has been the purpose of this investigation to study and compare the amylases produced by fungi requiring different ranges of H-ion concentration for growth. An attempt has been made to determine the effect of acidity and alkalinity upon the secretion and accumulation of the enzyme. Further, an endeavor has been made to determine whether the enzymes produced under these conditions have similar activities in buffered solutions covering a range of H-ion concentrations, and whether there is any correlation between the optimum for activity and the optimum for secretion and accumulation. It was considered unnecessary to determine the effect of the H ions apart from the other ions in the buffered solution, since the purpose was not to determine the absolute optimum H-ion concentration for the activity of the enzymes but merely to produce a set of similar



conditions under which amylolytic action could be studied. Thus, a means was furnished for determining whether the amylase produced by organisms requiring different H-ion concentrations for growth would be similar.

Whether the enzymes produced by one fungus or different fungi under widely divergent environmental conditions have similar properties might assist in explaining such problems in parasitism as host specialization and the establishment of strains, and in saprophytism, specialization as to habitat.

### SURVEY OF LITERATURE

The influence of acids and alkalis upon the activities of various enzymes was early noticed by different investigators. Pasteur, in 1879, observed the effect of acidity upon the alcoholic fermentation of wines and beers. Probably the first careful work on the influence of small quantities of acids and alkalis upon amylolytic action was done by Kjeldahl ('79). He showed that small quantities of mineral and organic acids increased the saccharogenic activity of a malt extract, while large amounts caused retardation.

A voluminous literature has been developed, since that time, upon this aspect of amylolytic activity. The early work relating to the influence of acids and alkalis is very conflicting, both acid and neutral conditions being given as producing optimum activity. Many of these inconsistencies have arisen from a lack of means for determining the exact concentration of H and OH ions in the solution used in the experiment, and thus the data are often difficult to interpret. For this reason, the results of the early investigators have been omitted from the following discussion. An adequate review of the more important literature may be found in the articles by Sherman and his associates ('15) and in the texts by Bayliss ('14), Euler ('12), and Green ('99).

The perfection of methods for measuring H ion concentration, as discussed by Sørensen ('09) and Michaelis ('14), has been a means of obtaining valuable data in regard to the effects of H ions upon enzyme action. The establishment of definite  $P_H$  ranges for the indicators has cleared up many discrepancies. Thus, in the work of Maquenne and Roux ('06), the maximum

initial activity of malt amylase was found to be in a solution neutral to methyl orange, but a solution alkaline to this indicator showed maximum total digestion of starch to maltose and glucose. Fernbach and Wolff ('06), working along the same line, found maximum amyloclastic and saccharogenic activities in solutions neutral to methyl orange. A secondary phosphate added to these solutions caused a depression of activity, while a primary phosphate produced either no effect or slight activation. The latter effect was ascribed to a failure to secure complete neutrality in the control solution. An analysis of their conclusions will show that the results are compatible with more recent work, since the range of color change in methyl orange is from about  $P_H$  3 to  $P_H$  5.

Kellerman ('03) studied the effects of various chemical agents upon the activity of Taka diastase by determining the reduction of the solutions with Fehling's solution. He concluded that at a concentration of N/10 all of the inorganic acids employed completely checked the activity. A dilution of N/1000 gave marked acceleration. The results with malt diastase were somewhat different in that acceleration did not occur until an N/5000 dilution was reached. Organic acids gave, in general, results similar to inorganic acids, but malic and acetic acids did not completely check hydrolysis at N/10 dilution. Acetic acid gave no acceleration until a dilution of N/2500 was reached. These differences were not explained by the author, but were, no doubt, due to differences in the ionization of the acids. Without any exception, the alkalis used seemed to be detrimental or slightly so, even up to N/10,000 dilution.

Cole ('03) reached the following conclusion as to the activity of ptyalin: "The hydrolysis of starch is accelerated by the presence in the solution of electro-negative ions (anions) other than OH ions and depressed by electro-positive ions (kations) and by hydroxyl ions." For example, the chlorine ion in HCl is the factor which increases the action, and in low concentration of acid the depression due to the H ions is not sufficient to show itself against the acceleration of the chlorine ions. Although his conclusions seem to be based upon insufficient data in some instances and his interpretations do not altogether agree with recent researches, he demonstrated that a low concentration of

acid increased the action of ptyalin, whereas larger amounts produced inhibition. He also noticed a difference in the accelerating action of acids in the presence of salts, an observation which was earlier reported by Wood ('94).

The effect of the OH-ion concentration in NaOH and  $\text{Na}_2\text{CO}_3$  solutions upon the saccharogenic power of three different amylases, Taka, saliva, and an extract of swine pancreas, was determined by Quinan ('09). Although no data on the exact OH-ion concentration were presented, the results are worthy of notice in that they show differences in the activities of the amylases employed. Using 100 mgs. of Taka diastase in 100 cc. of a 1 per cent starch solution and allowing the digestion to proceed 18 hours at  $36^\circ \text{C}$ ., he found the critical hydroxyl-ion concentration, viz., the concentration at which merely a trace of sugar was present, in the solution containing 2 cc. of N/10 NaOH or 6 cc. of N/10  $\text{Na}_2\text{CO}_3$ . Under the same conditions 1 cc. of saliva acting for 15 hours was found to yield a trace of sugar in solutions containing 1 cc. of N/100 NaOH or 4 cc. of N/100 of  $\text{Na}_2\text{CO}_3$ . Similarly, in 10 hours, 1 cc. of an extract of swine pancreas yielded merely a trace of sugar in solutions containing approximately 3 cc. N/10 NaOH and less than 10 cc. of N/10  $\text{Na}_2\text{CO}_3$ . The differences in the effects of these alkalis upon the various diastase activities, he stated, were due to differences in dissociation and thus in the amounts of OH ions present.

Although the investigations by Sørensen ('09) and those later by Michaelis ('14) and his associates were not upon amylase specifically, some of the results deserve mention in this connection. Sørensen, studying the factors influencing the activity of catalase and pepsin, concluded that the activity varied according to the actual H-ion concentration and not to the titratable acidity. He also found that there existed one optimum H-ion concentration for each enzyme, regardless of the acid used, and this optimum was dependent upon the time and the temperature at which the enzyme was allowed to act upon the substrate. Thus, after a few minutes in the case of invertase, inversion occurred most rapidly at  $\text{P}_\text{H}$  3.68 and as the time increased the optimum was shifted toward the neutral point until, in 32 minutes, the optimum was  $\text{P}_\text{H}$  4.8. He further noted the similarity of the H-ion curve to the temperature curve in enzymatic activity.

Michaelis, publishing with Davidson and later with Peckstein, established definite H-ion concentration optima for various enzymes and also showed that there is an optimum zone rather than a sharply defined point. Michaelis and Peckstein found that ptyalin formed complex combinations with many neutral salts. The affinity for various anions varied greatly, being greatest with the nitrate ion, slightly less with chlorine and bromine, and very little with the sulphate, acetate, and phosphate ions. Each one of these diastase-complexes was found to be a characteristic compound, the fermentative action upon starch being noticeably different and the H-ion concentration optima with regard to activity also varying. The chlorine complex produced the most reactive combination, the nitrate slightly less, and the sulphate, acetate, and phosphate least of all. The H-ion concentrations producing optimum activity were found to be as follows:

|                         |                              |
|-------------------------|------------------------------|
| Nitrate-diaastase.....  | P <sub>H</sub> 6.9           |
| Chlorine-diaastase..... | P <sub>H</sub> 6.7           |
| Phosphate-diaastase }   | ..... P <sub>H</sub> 6.1-6.2 |
| Sulphate-diaastase }    |                              |
| Acetate-diaastase }     |                              |

A series of investigations carried on by Sherman and his associates has been helpful in indicating a field for the study of the properties and conditions for the action of amylases when purified materials are used.

Sherman and Thomas ('15) determined the H-ion concentration most favorable for the activity of malt amylase at 40° C. The weak and strong acids and the acid phosphates of sodium and potassium all showed an optimum activation in solutions having nearly the same actual acidity, this varying from P<sub>H</sub> 4.2 to 4.6. With amounts of strong acid above the optimum concentration, the depression of action was greater than with corresponding excesses of weak acids. The activity was determined by the reduction of Fehling's solution and also by the starch iodide method as in Wohlgemuth's modification. The results with these two methods were found to differ. The amylolytic action, as measured by the Wohlgemuth method, reached an optimum at a concentration of the activating agent (either salt or acid) much below that which gave optimum

saccharogenic action as determined by the reduction of Fehling's solution.

In a later paper by Sherman and Walter ('17), the action of a very concentrated preparation of malt amylase on purified soluble starch was investigated by observing the rate of formation of maltose in neutral solutions without electrolytes and in solutions containing regulated amounts of  $\text{HCl}$ ,  $\text{H}_3\text{PO}_4$ , and  $\text{KH}_2\text{PO}_4$ . When the optimum H-ion concentration,  $C_{\text{H}} = 1 \times 10^{-4}$ , was reached, the action of the enzyme was increased at all stages. This optimum was the same for all of the above electrolytes. The greater the concentration of the enzyme, the less the effect of the electrolyte.

Sherman, Thomas, and Baldwin ('19) recently studied purified amylases obtained from three different sources, in order to determine the optimum H-ion concentration and to establish the "limits of H-ion concentration within which any enzymic activity is shown and the form of the curve representing the activities at all concentrations of H-ions between these limits." Pancreatic and malt amylase and the amylase of *Aspergillus Oryzae* were chosen as representative of the enzyme as it occurs in higher animals, higher plants, and fungi, respectively. The activity of the enzymes was studied in solutions having a range of approximately  $P_{\text{H}}$  2-10. These solutions contained  $\text{H}_3\text{PO}_4$  and  $\text{NaH}_2\text{PO}_4$ ,  $\text{Na}_2\text{HPO}_4$  and  $\text{Na}_2\text{CO}_3$ , and, in some cases,  $\text{NaH}_2\text{PO}_4$  or  $\text{Na}_2\text{HPO}_4$ . The action of the enzyme upon the substrate took place at  $40^\circ \text{C}$ . and the analyses were made with Fehling's solution. The results showed that pancreatic amylase was active within a range of  $P_{\text{H}}$  4-10, the optimum being at about  $P_{\text{H}}$  7. Malt amylase was active between  $P_{\text{H}}$  2.5-9, with an optimum at  $P_{\text{H}}$  4.4-4.5, and the amylase of *Aspergillus Oryzae* showed activity between  $P_{\text{H}}$  2.6-8, with an optimum at about  $P_{\text{H}}$  4.8. It was thus shown that the amylase of malt and *Aspergillus Oryzae* possessed similar saccharogenic powers in the solutions used in the experiment, while pancreatic amylase not only had a different range in activity but also possessed a higher optimum. The influence of the electrolyte, as distinguished from the H-ion concentration alone, seemed greatest in the case of pancreatic amylase and least in the case of the amylase of *Aspergillus Oryzae*.

A study of the activity of a purified malt amylase in buffered solutions of varying H-ion concentrations was also made by Adler ('16). The enzyme was allowed to act upon the substrate for one hour at 20° C. and the activity measured by the reduction of Fehling's solution and also by the starch iodide reaction. As a result of these investigations, he found that there was an optimum point of  $P_H$  4.9 and that an increase or decrease in the H-ion concentration resulted in a rapid suppression of action. It was also shown that neutral ions were not without effect, although they exerted an influence less than the Hions. Determinations with polarized light of the reducing substances in the solutions gave unsatisfactory results, since the solutions were not always clear. Adler believed that a direct relation between the reduction of Fehling's solution and the amount of polarization did not always exist, thus indicating the possibility of a difference in the effect of the H-ion concentration upon the dextrin production.

In this connection, the work of Chrzaszcz and Joscht ('17) may be mentioned. They concluded that malt amylase was composed of two clearly defined enzymes, a starch-dissolving enzyme, a sugar-producing enzyme, and a starch-dextrin enzyme, which was considered a resultant of the two foregoing enzymes. The iodine reaction corresponded first to the activity of the starch-dissolving and then to the sugar-producing enzyme. However, the correspondence was mostly to the soluble portion. The hydroxides, in general, were found to be unfavorable to the production of sugar in the enzymic reaction, but favorable to the starch-dissolving and dextrin-forming complexes.

Using a modified Lintner method, Falk, McGuire, and Blount ('19) studied some vegetable enzymes in fresh, vacuum-dried and air-dried material in relation to acidity, at 37° C., acting for 2 hours. Well-defined optima were obtained at about  $P_H$  6 with cabbage, carrot, and white turnip juices. With yellow turnip juice, the optimum action extended from  $P_H$  4 to 7. The H-ion concentrations of all the juices from fresh and dehydrated material prepared according to the method described were found to be about  $P_H$  6. Thus, it is seen that the optimum H-ion concentration for the amylases coincided with the natural H-ion concentration of the juices. Dehydration decreased the action in every case.

From the preceding discussion, it is evident that in recent years the effect of the H-ion concentration upon amylolytic activity has been carefully studied by several investigators. Although the results are not voluminous, they have shown that the reaction of the solution has a marked effect upon enzymic activity. They have shown, further, that amylases from different sources may react differently in this respect.

Scarcely any investigations have been undertaken with a view to determining the influence of H- and OH-ion concentration upon the formation of amylase in the organism. In most of the work on the effect of various conditions upon the secretion of amylase, no mention has been made of the concentration of the H ions coincident with the varying conditions. It is not pertinent to the present discussion to review such works as those by Katz, Dox, Hasselbring, Saito, and others who have studied the relation of organic substances to the production and secretion of amylase. However, it is worthy of note that in many cases neutral, alkaline, and acid substrates were used and that no attempt was made to control the H-ion concentration during the experiment.

The results obtained on the influence of inorganic substances on amylolytic activity are likewise often difficult to interpret. Robbins ('16), in an extended study of the secretion of amylase by *Penicillium Camembertii*, determined the effect of single salts and the absence of salts in a nutrient solution. The fungus was grown for two weeks at 25° C. in various solutions containing an approximately constant amount of starch. At the end of this time, in the single salt cultures, the mycelium was filtered off, the acidity or alkalinity determined by means of methyl orange and phenolphthalein, and the starch and dextrins undigested in the solution measured by a new method which was based on their insolubility in an acidified aqueous alcoholic solution. The salts in the single salt series, with the exception of the acid phosphates, were all neutral salts, so it was very likely that the H-ion concentration at the beginning of growth was the same, since the highest purity chemicals were employed. The reaction of the solution after the growth of the fungus was found to be alkaline to methyl orange and acid to phenolphthalein, which might indicate a variation from about  $P_H$  4-8.

and thus would embody acid, neutral, and alkaline conditions. Under these conditions, he found that potassium salts inhibited digestion more than sodium salts, that potassium and calcium did not seem to be connected with amylase formation, and that there appeared to be an intimate relation between nitrogen and amylase formation. In the nutrient solutions, where salt substitutions were made, the H-ion concentrations, when determined by the method outlined in this paper, were found to vary from  $P_H$  3.3 to 5.4. After the growth of the fungus, the H-ion concentration undoubtedly was shifted, a fact which might have been significant in explaining the results. It is worthy of note that a marked difference was observed by Robbins between the speed with which *Aspergillus Oryzae* and *Penicillium Camembertii*, on the one hand, and *Fusarium* sp. and *Mucor Rouxii*, on the other, digest soluble starch in the absence of all nutrients. The former had a very slow rate while the latter showed fairly rapid digestion.

That invertase formation in yeasts is dependent upon acidity has been shown by Euler and Svanberg ('19). Optimum accumulation was effected at an H-ion concentration of  $P_H$  5-6 and a concentration of  $P_H$  2 was found to be destructive.

Further, Euler and Emberg ('19) attempted to determine the influence of acidity and alkalinity upon enzyme formation by a bottom yeast and the adaptation of these living cells to nutrient solutions. They showed that the maximum H- and OH-ion concentrations at which the cells reproduce themselves or exhibit enzymic relations could be modified by adaptation.

## METHODS AND MATERIALS

### ORGANISMS

The fungi used in this investigation were selected with a view to obtaining a group of parasitic organisms which differ in optimum growth with reference to the H-ion concentration of the medium. One organism producing maximum growth in acid media, one growing well in alkaline media, and one requiring either acid or alkaline media were thus chosen. *Colletotrichum Gossypii* (Southworth), *Penicillium italicum*,<sup>1</sup> and *Fusarium* sp.

<sup>1</sup> Determined by Dr. Chas. Thom.



were taken as representative of these conditions. Duggar, Severy, and Schmitz ('17) have shown that *C. Gossypii* is a form which produces growth on media having an alkaline reaction and also that it shifts towards the alkaline side the reaction of certain sugar-containing media upon which it is growing. The fact that *P. italicum* grows abundantly upon citrus fruits whose reaction varies from  $P_H$  2.2 to 4.1 seemed to indicate that it was an organism which could be used to represent activities occurring on the acid side. The culture of *Fusarium* sp. was isolated from cotton and is the same organism as used by Webb ('19). He found that the spores had a wide range for germination in relation to H-ion concentration, varying from  $P_H$  2.8 to 10+ in the NaOH- $H_3PO_4$ -mannite solution used. By employing these forms, it was thought that the amylase formation under different H-ion concentrations could be studied.

The cultures from which spores were obtained for the subsequent inoculations were on media which produced abundant sporulation at room temperature. A synthetic medium prepared according to the following formula<sup>1</sup> was used for *C. Gossypii*.

|                                       |           |
|---------------------------------------|-----------|
| MgSO <sub>4</sub> .....               | .25 gms.  |
| K <sub>2</sub> HPO <sub>4</sub> ..... | .25 gms.  |
| Peptone .....                         | 10.0 gms. |
| Glucose .....                         | 20.0 gms. |
| Agar .....                            | 15.0 gms. |
| Water .....                           | 1000 cc.  |

*Fusarium* sp. was grown on potato agar made according to the method described by Duggar, Severy, and Schmitz ('17), while Czapek's solution containing starch as the source of energy and 1.5 per cent agar was found to produce abundant spores in the case of *P. italicum*.

*Spore suspension*—The spores used in making the subsequent inoculations for the enzyme studies were from cultures about 14 days old. A uniform suspension was obtained by putting the spores from the culture in 5 cc. of sterile doubly distilled water. A microscopical examination by means of a hanging drop was made of a loopful of this suspension. An average of 6 spores to a field under low power was taken as the standard.

<sup>1</sup> This formula was furnished by Prof. Barre, of Clemson College, S. C. The original citation is unknown to the author.

## PREPARATION OF CULTURES FOR ENZYME STUDIES

*Chemicals and glassware.*—In all of the following experiments the highest purity chemicals and water redistilled from a trace of potassium permanganate and a few drops of sulphuric acid were used unless otherwise specified. The doubly distilled water gave an H-ion concentration of  $P_H$  5.2–5.6. The glassware was chemically cleaned and rinsed with distilled and doubly distilled water except in the experiments for the sugar determinations where distilled water was employed.

The fungi were grown in 300-cc. Pyrex flasks, and the culture solution was prepared according to the following modification of Czapek's solution:

|                                       |      |      |
|---------------------------------------|------|------|
| MgSO <sub>4</sub> .....               | .5   | gms. |
| KH <sub>2</sub> PO <sub>4</sub> ..... | 1.0  | gms. |
| KCl.....                              | .5   | gms. |
| FeSO <sub>4</sub> .....               | .01  | gms. |
| NaNO <sub>3</sub> .....               | 2.0  | gms. |
| Soluble starch.....                   | 5.0  | gms. |
| Cane sugar.....                       | .05  | gms. |
| Water.....                            | 1000 | cc.  |

The monobasic potassium phosphate was extremely acid and therefore it was recrystallized until a solution of M/15 gave a reaction of  $P_H$  4.5. Starch was added as the source of energy, since Dox ('10) and also later investigators have shown that its presence in culture solutions increases the production of amylase. It has been shown, further, that the presence of a trace of sugar, which increases the initial growth, is beneficial to the formation of amylase.

A range of  $P_H$  1.8–9.4 was obtained in this culture solution by the addition of regulated amounts of N/20 KOH and N/5 HCl according to the method described by Karrer and Webb ('20). From their curve, the amounts of acid and alkali necessary to bring the solution to the required H-ion concentration can be computed. The solutions containing 5 and 10 cc. of N/20 KOH all showed a slight precipitate. Fifty flasks containing a solution of the same H-ion concentration constituted a series. The flasks were inoculated with a loopful of spore suspension, one uninoculated flask being kept as a control. Five series with the culture solution adjusted to  $P_H$  3.0,  $P_H$  4.5,  $P_H$  7.0,

$P_H$  8.2, and  $P_H$  9.2, respectively, were studied in the case of *Fusarium* sp.; for *Colletotrichum Gossypii*, solutions of  $P_H$  4.5,  $P_H$  7.0,  $P_H$  8.2, and  $P_H$  9.2 were employed; and *Penicillium italicum* was grown in solutions of  $P_H$  3.0 and  $P_H$  4.5. The flasks were placed in the dark at a constant temperature of 28° C. for 2 weeks. At the end of this time, dry weight determinations were made and the final H-ion concentration of the culture media taken. Dry weight determinations were made of the fungous mats in 10 of the flasks. The material was poured upon a weighed filter-paper in a Gooch funnel, thoroughly washed with doubly distilled water, and dried by means of suction. These mats were first allowed to dry in the air for 24 hours and then over  $CaCl_2$  in a desiccator for about one week. After this the final weighings were taken. The remaining cultures were filtered and dried in a similar manner.

The H-ion concentration of the control culture solution and of the solution upon which the fungus had been grown was taken according to the method of Clark and Lubs ('17). After 1 per cent toluene was added to the culture solution, it was stored in a refrigerator for subsequent enzyme determinations, which were made within 1-3 days.

### ENZYME STUDIES

Amylolytic activity was studied with the fungous mycelium and with the culture solution.

*Preparation of materials.*—A series of buffer solutions of different H-ion concentrations in which enzyme activity could be tested were prepared by varying the amounts of N/5 NaOH added to a given volume of  $H_3PO_4$ , according to the orthophosphoric acid titration curve given by Clark and Lubs ('17). Solutions of  $P_H$  3, 4, 6, 7, 8, 9, and 11 and doubly distilled water were used in a series. With the exception of the  $P_H$  4 solution, the H-ion concentration coincided with that calculated from the curve. In the case of  $P_H$  4, the critical point as seen in the curve, is so sharply defined that the addition of a small fraction of a cubic centimeter of NaOH resulted in a decided change in the H-ion concentration. Thus, this solution was usually about  $P_H$  5.2.

A 5 per cent soluble starch solution, which was to be added to the above as a substrate, was prepared by mixing the requisite amount of Lintner's soluble starch (Merck) and water and refluxing for about 3 hours. One per cent of toluene was added to the stock solution as a preservative.

*Extracellular amylase.*—It was found in preliminary experiments that the total volume of the nutrient solution remaining in the various flasks after the growth of the fungus averaged about 40 cc. in all the series, so it was unnecessary to bring the total volume of the solution to a definite volume.

In studying the extracellular amylase, 39 cc. of each of the above-described solutions were placed in 100-cc. Erlenmeyer flasks. To each of these, 1 cc. of the starch paste and 10 cc. of culture solution were added, thus making the total volume 50 cc. One per cent toluene was used as a preservative. The solutions were then incubated at 28° C. for 24 hours. A smaller quantity than 10 cc. of culture solution containing the excreted enzyme was insufficient to produce marked activity in some cases, and a greater amount often caused too much variation in the final H-ion concentration of the solution. Further, an incubation period of 6–12 hours was found to give such low diastatic values that a longer period of incubation was employed. A control series was set up by adding 10 cc. of the culture solution, which had been inactivated by heating in a boiling water-bath for 15 minutes and then made up to the original volume, to solutions of the above H-ion concentrations. In this manner the effect of the H-ion concentration upon the reagents, and also the reducing power of the enzyme solution was determined. A determination was made of the amount of reduction occurring in the enzyme solution alone. This was found not to vary during the course of the experiment.

*Method of determining the enzymic action.*—At the end of 24 hours, the saccharifying power of the enzyme was tested according to the following method. Ten cc. of each of the buffered solutions and distilled water were accurately pipetted into each of two 50-cc. centrifuge tubes and made neutral to phenolphthalein by adding either NaOH or  $H_3PO_4$ . The tubes were then plunged into boiling water for 10 minutes in order to inactivate the enzyme. The same procedure was followed for the inacti-

vated series. Five cc. of Fehling's solution, prepared according to the standard formula, were added to four of these tubes at a time, and the tubes immersed immediately in an actively boiling water bath for 10 minutes. The amount of reduction was determined by the Bertrand method as described by Shaffer ('14), N/50  $\text{KMnO}_4$  being used in the titrations. The results were all obtained in duplicate. These differed from each other by not more than .03 cc. of N/50  $\text{KMnO}_4$ . In all cases the H-ion concentrations of the control and active solutions were taken at the beginning and end of the period of incubation. These were found to remain constant during the time of the experiment. The H-ion concentration was shifted somewhat in several instances after the enzyme dispersion was added, this being due to the presence of alkali or to the presence of salts which affected the original buffer solution.

*Intracellular amylase.*—The dry fungous mats were powdered in a mortar, and 2.5 gms. were again ground with about an equal amount of powdered Pyrex glass. The enzyme was extracted from this mixture with 125 cc. of doubly distilled water for 12 hours. An extraction of from 1 to 4 hours was found to yield a dispersion of weaker amylolytic activity. This dispersion was filtered and its activity tested at different H-ion concentrations in a manner similar to that discussed for extracellular amylase, except that 44 cc. of the buffer solution, 5 cc. of enzyme extract, and 1 cc. of 5 per cent soluble starch were used. The enzyme activity was tested exactly as described above.

#### EXPERIMENTAL DATA AND DISCUSSION

The experimental data will be discussed under the following topics:

(1) An analysis of the cultures, embodying the effect of the H-ion concentration upon the dry weight of the fungus and the effect of the growth of the fungus upon the final H-ion concentration of the culture solution, together with notes on some cultural characteristics of the organisms.

(2) The influence of H-ion concentration of the  $\text{NaOH-H}_2\text{PO}_4$  solutions upon the activity of the amylase produced in culture solutions having different H-ion concentrations.

(3) The effect of the H-ion concentration of the culture medium upon the accumulation of amylase.

*Analysis of the cultures.*—From the following table (table I) it will be seen that the amounts of mycelium of the cultures selected for enzyme determinations when expressed on a dry weight basis were practically the same at the different H-ion concentrations of the nutrient solutions. A small amount of growth is also noticeable in these cases. This, however, was to

TABLE I

DRY WEIGHT DETERMINATIONS OF ORGANISMS EMPLOYED WITH THE INITIAL AND FINAL H-ION CONCENTRATION OF THE CULTURE SOLUTION

| Organism                           | Ser. | Dry wts.<br>in gms.* | Amts. of N/5 HCl and N/20<br>KOH added in 50 cc. of<br>nutr. sol. |              | P <sub>H</sub> of nutrient<br>solution |       |
|------------------------------------|------|----------------------|---|--------------|--|-------|
|                                    |      |                      | Cc. N/5 HCl   | Cc. N/20 KOH | Initial                                | Final |
| <i>Fusarium</i> sp.                | I    | .0973                | 0.5   | ....         | 3.0                                    | 7.2   |
|                                    | II   | .0969                | ....  | ....         | 4.5                                    | 7.8   |
|                                    | III  | .1061                | ....  | 5.0          | 7.0                                    | 9.0   |
|                                    | IV   | .1010                | ....  | 10.0         | 8.2                                    | 9.0   |
|                                    | V    | .1023                | ...   | 20.0         | 9.2                                    | 9.2   |
| <i>Colletotrichum<br/>Gossypii</i> | VI   | .1069                | ....  | ....         | 4.5                                    | 6.7   |
|                                    | VII  | .0976                | ....  | 5.0          | 7.0                                    | 7.9   |
|                                    | VIII | .0982                | ....  | 10.0         | 8.2                                    | 8.4   |
|                                    | IX   | .0817                | ....  | 20.0         | 9.2                                    | 8.6   |
| <i>Penicillium<br/>italicum</i>    | X    | .0897                | 0.5   | ....         | 3.0                                    | 6.0   |
|                                    | XI   | .0909                | ....  | ....         | 4.5                                    | 6.3   |

\* The average amount of growth produced in one flask containing 50 cc. of culture solution.

be expected since the amount of mycelium produced by these organisms is never so luxuriant as that produced by *Aspergillus*, *Botrytis*, and other fungi. Dox ('10) has also pointed out that less growth resulted for the organisms used in his experiments in a nutrient solution containing starch than in one containing some other carbohydrate. With all the cultures of *Fusarium* sp., growth appeared at the end of about 4 days. The mycelium grew on the surface of the solution in a fluffy mat and the spores were produced at all H-ion concentrations. At a concentration

of  $P_H$  2.0 no growth occurred, and the limit on the alkaline side was found to be beyond  $P_H$  9.2. Thus, the growth range followed the range for spore germination as determined by Webb ('19), this being from  $P_H$  2.8 to 10.0+.

With the exception of the  $P_H$  9.2 culture solution, the reaction of all the culture solutions, with starch as the source of energy, was changed during the growth of the fungus, this shift being toward increased alkalinity. The greater the acidity the greater the change produced. Thus, the reaction of the  $P_H$  3.0 culture solutions was shifted to 7.0 while that of  $P_H$  8.2 was shifted to 9.0.



Fig. 1. Action of extracellular (—) and intracellular (---) amylase produced by *Fusarium* sp. grown in Czapek's solution of  $P_H$  3.0.

*Colletotrichum Gossypii* produced visible growth at the end of 3 to 4 days at favorable H-ion concentrations. Abundant spores were produced in the cultures, and the mycelium from the first was very gelatinous and attached to the bottom of the flask. The growth range was found to be from  $P_H$  3.0-4.5 to 9.2 or beyond for the production of a fair quantity of mycelium. The final H-ion concentration of the solution, although shifted toward the alkaline side in all but one instance, gave results, in

the corresponding solutions, much less than those of *Fusarium*. The greatest shift was from  $P_H$  4.5 to 6.7 in natural Czapek's solution. The alkalinity of the culture solution with an initial reaction of  $P_H$  9.2 was somewhat lessened during the growth of the fungus, since the final reaction was  $P_H$  8.6.

*Penicillium italicum* was found to have a more limited growth range with respect to the reaction of the culture solution than

either of the other organisms. The best results were obtained under acid conditions from  $P_H$  2.5 to 4.5. At  $P_H$  8.0 only a few hyphae were produced from the spores. Visible growth appeared at the end of about 4 days, and the mycelium grew on the surface of the liquid forming a rather thin, felt-like mass. No spores were produced in the culture solution having a concentration of  $P_H$  3.0, but there was abundant production in most of the natural Czapek's solution cultures of  $P_H$  4.5.

*Amylase activities.*—As stated above, the results of the experiments on amylase activity will be discussed from two standpoints: the effect of the buffer solution of various H-ion concentrations upon the activity of the amylase produced in the different culture solutions, and the effect of the H-ion concentration of the culture medium upon its accumulation in the mycelium and the culture solution. The data will be presented in the form of tables, also of curves where the abscissae are the H-ion concentrations of the buffered solutions in which enzyme activity was measured, and the ordinates, the cubic centimeters of the  $N/50$   $KMnO_4$  solution which represent the relative amounts of starch hydrolyzed and therefore the extent of enzyme production. As shown in tables II-IV, the results of the inactivated or control

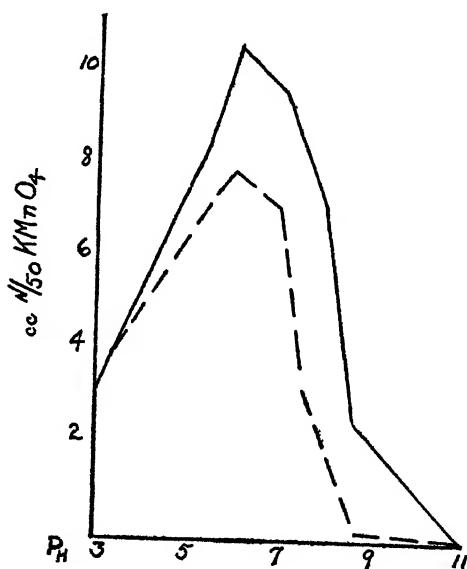


Fig. 2. Action of extracellular (—) and intracellular (---) amylase produced by *Fusarium* sp. grown in Czapek's solution of  $P_H$  4.5.

series were subtracted from the active series in order to produce these amounts. The extracellular amylase curve denotes hydrolysis with 2 cc. of culture solution and the curve of intracellular amylase with 1 cc. of 2 per cent mycelium dispersion or .02 gm. of the powdered mycelium.



Since the dry weight determinations of Series 1-11 inclusive were equal within the limits of experimental error and the total amounts of culture solution remaining in the flasks after the

TABLE II

SHOWING THE ACTIVITY OF INTRA- AND EXTRACELLULAR AMYLASE PRODUCED BY FUSARIUM SP. WHEN GROWN IN CZAPEK'S SOLUTION HAVING INITIAL H-ION CONCENTRATIONS OF  $P_H$  3.0 AND 4.5

Initial H-ion concentration of Czapek's solution

|                       | $P_H$ 3.0             |                   |         |                  | $P_H$ 4.5             |                   |         |                  |
|-----------------------|-----------------------|-------------------|---------|------------------|-----------------------|-------------------|---------|------------------|
|                       | $P_H$ of buffer sol.* | Cc. N/50 $KMnO_4$ |         |                  | $P_H$ of buffer sol.* | Cc. N/50 $KMnO_4$ |         |                  |
|                       |                       | Total             | Control | Amylase activity |                       | Total             | Control | Amylase activity |
| Extracellular amylase | 3.0                   | 2.2               | 0.4     | 1.8              | 3.0                   | 4.3               | 1.3     | 3.0              |
|                       | 5.3                   | 6.7               | 0.5     | 6.2              | 5.2                   | 9.8               | 1.3     | 8.5              |
|                       | 6.0                   | 7.1               | 0.4     | 6.7              | 6.0                   | 12.2              | 1.4     | 10.8             |
|                       | 7.0                   | 6.0               | 0.4     | 5.6              | 7.0                   | 11.0              | 1.3     | 9.7              |
|                       | 8.0                   | 4.0               | 0.6     | 3.4              | 8.0                   | 8.5               | 1.5     | 7.0              |
|                       | 8.6                   | 1.3               | 0.5     | 0.8              | 8.6                   | 4.1               | 1.5     | 2.6              |
|                       | 11.0                  | 0.7               | 0.5     | 0.2              | 11.0                  | 1.5               | 1.6     | 0.0              |
|                       | 7.2†                  | 6.5               | 0.5     | 6.0              | 7.2†                  | 10.5              | 1.5     | 9.0              |
| Intracellular amylase | 3.0                   | 9.2               | 2.8     | 6.4              | 3.2                   | 6.6               | 2.6     | 4.0              |
|                       | 5.6                   | 11.1              | 2.8     | 8.3              | 5.2                   | 10.1              | 2.9     | 7.2              |
|                       | 6.0                   | 13.2              | 3.0     | 10.2             | 6.0                   | 10.8              | 2.9     | 7.9              |
|                       | 7.0                   | 11.8              | 2.8     | 9.0              | 6.9                   | 10.1              | 2.9     | 7.2              |
|                       | 7.7                   | 9.2               | 2.8     | 6.4              | 7.5                   | 6.4               | 2.9     | 3.5              |
|                       | 8.2                   | 6.1               | 2.9     | 3.2              | 8.7                   | 2.8               | 2.6     | 0.2              |
|                       | 11.0                  | 3.0               | 2.8     | 0.2              | 11.0                  | 2.6               | 2.6     | 0.0              |
|                       | 6.0†                  | 13.3              | 2.8     | 10.5             | 6.1†                  | 9.9               | 2.5     | 7.4              |

\* H-ion concentration of the buffer solution in which enzyme activity was measured.

† Doubly distilled water was substituted for the buffer solution.

growth of the fungus were the same, the tables and curves can be compared directly. The amounts of enzyme produced in the culture solution according to the tables will, thus represent

TABLE III  
SHOWING THE ACTIVITY OF INTRA- AND EXTRACELLULAR AMYLASE PRODUCED BY FUSARIUM SP. WHEN GROWN IN CZAPEK'S SOLUTION WITH H-ION CONCENTRATIONS OF  $P_H$  7.0, 8.2, AND 9.2

| Initial H-ion concentration of Czapek's solution |                            |         |                  |                                |                            |         |                  |                            |                  |
|--|----------------------------|---------|------------------|--------------------------------|----------------------------|---------|------------------|----------------------------|------------------|
| $P_H$ 7.0  |                            |         |                  | $P_H$ 8.2                      |                            |         |                  | $P_H$ 9.2                  |                  |
| P <sub>H</sub> of buffer sol.*                   | Cc. N/50 KMnO <sub>4</sub> |         |                  | P <sub>H</sub> of buffer sol.* | Cc. N/50 KMnO <sub>4</sub> |         |                  | Cc. N/50 KMnO <sub>4</sub> |                  |
|  | Total                      | Control | Amylase activity |                                | Total                      | Control | Amylase activity | Total                      | Amylase activity |
| Extracellular                                    | 3.0                        | 0.6     | 0.6              | 3.2                            | 1.1                        | 0.8     | 0.3              | 0.8                        | 0.0              |
|  | 5.0                        | 1.6     | 0.6              | 5.7                            | 2.6                        | 0.9     | 1.7              | 2.4                        | 1.7              |
|  | 6.8                        | 2.1     | 0.6              | 6.2                            | 2.9                        | 0.9     | 2.0              | 2.5                        | 1.6              |
|  | 7.1                        | 1.4     | 0.5              | 7.0                            | 1.9                        | 0.8     | 1.1              | 2.2                        | 1.4              |
|  | 8.0                        | 0.6     | 0.6              | 8.0                            | 1.1                        | 0.9     | 0.2              | 1.6                        | 0.8              |
|  | 9.0                        | 0.6     | 0.6              | 9.0                            | 0.9                        | 0.9     | 0.0              | 0.8                        | 0.0              |
|  | 11.0                       | 0.6     | 0.6              | 11.0                           | 0.9                        | 0.9     | 0.0              | 0.8                        | 0.0              |
|  | 7.6†                       | 1.6     | 0.6              | 7.8†                           | 1.3                        | 0.8     | 0.5              | 1.6                        | 0.8              |
| Intracellular                                    | 3.2                        | 4.4     | 3.4              | 3.1                            | 4.8                        | 3.5     | 1.3              | 4.7                        | 2.3              |
|  | 4.4                        | 9.5     | 3.6              | 4.4                            | 5.4                        | 3.5     | 1.9              | 6.3                        | 3.7              |
|  | 6.0                        | 9.6     | 3.7              | 6.1                            | 7.3                        | 3.4     | 3.9              | 6.8                        | 4.3              |
|  | 6.9                        | 7.8     | 3.4              | 7.0                            | 5.4                        | 3.4     | 2.0              | 5.5                        | 3.2              |
|  | 7.6                        | 5.7     | 3.5              | 8.0                            | 3.6                        | 3.6     | 0.2              | 4.7                        | 2.2              |
|  | 9.0                        | 3.5     | 3.5              | 9.0                            | 3.5                        | 3.5     | 0.0              | 3.7                        | 1.2              |
|  | 11.0                       | 3.5     | 3.5              | 11.0                           | 3.5                        | 3.5     | 0.0              | 2.5                        | 0.0              |
|  | 6.4†                       | 8.5     | 3.5              | 6.6†                           | 6.9                        | 3.4     | 3.5              | 5.6                        | 3.0              |

H-ion concentration of the buffer solution in which enzyme activity was determined. Doubly distilled water was substituted for the buffer solution.

TABLE IV

SHOWING THE ACTIVITY OF INTRA- AND EXTRACELLULAR AMYLASE  
PRODUCED BY COLLETOTRICHUM GOSSYPHII WHEN GROWN IN  
CZAPEK'S SOLUTION HAVING INITIAL H-ION CON-  
CENTRATIONS OF  $P_H$  4.5, 7.0, 8.2, AND 9.2

|                          |         | Initial H-ion concentration of Czapek's solution |                            |       |                    |                                      |                            |      |
|--------------------------|---------|--|----------------------------|-------|--------------------|--------------------------------------|----------------------------|------|
|                          |         | P <sub>H</sub> 4.5                               |                            |       |                    | P <sub>H</sub> 7.0                   |                            |      |
|                          |         | P <sub>H</sub> of<br>buffer<br>sol.*             | Cc. N/50 KMnO <sub>4</sub> |       |                    | P <sub>H</sub> of<br>buffer<br>sol.* | Cc. N/50 KMnO <sub>4</sub> |      |
| Total                    | Control |  | Amylase<br>activity        | Total | Control            |                                      | Amylase<br>activity        |      |
| Extracellular<br>amylase | 3.0     | 7.1  | 0.5                        | 6.6   | 3.0                | 14.7                                 | 0.9                        | 13.8 |
|                          | 5.2     | 9.3  | 0.5                        | 8.8   | 5.1                | 14.9                                 | 0.7                        | 14.2 |
|                          | 6.0     | 9.4  | 0.4                        | 9.0   | 5.9                | 15.7                                 | 0.8                        | 14.9 |
|                          | 7.0     | 9.2  | 0.4                        | 8.8   | 7.0                | 13.8                                 | 0.8                        | 13.0 |
|                          | 8.0     | 5.9  | 0.4                        | 5.5   | 8.0                | 12.4                                 | 0.8                        | 11.6 |
|                          | 8.4     | 2.5  | 0.4                        | 2.1   | 8.5                | 1.4                                  | 0.9                        | 0.5  |
|                          | 11.0    | 0.5  | 0.5                        | 0.0   | 11.0               | 0.9                                  | 0.9                        | 0.0  |
|                          | 6.6†    | 10.0   | 0.5                        | 9.5   | 6.8†               | 13.7                                 | 0.7                        | 13.0 |
| Intracellular<br>amylase | 3.0     | 5.8  | 0.8                        | 5.0   | 3.0                | 13.2                                 | 0.4                        | 12.8 |
|                          | 5.2     | 9.5  | 0.7                        | 8.8   | 5.5                | 14.0                                 | 0.4                        | 13.6 |
|                          | 6.1     | 10.9   | 0.6                        | 10.3  | 6.0                | 15.5                                 | 0.4                        | 15.1 |
|                          | 7.0     | 9.8  | 0.8                        | 9.0   | 7.0                | 13.8                                 | 0.5                        | 13.3 |
|                          | 8.2     | 5.2  | 0.8                        | 4.4   | 8.0                | 12.0                                 | 0.4                        | 11.6 |
|                          | 8.4     | 2.8  | 0.7                        | 2.1   | 8.8                | 7.2                                  | 0.5                        | 6.7  |
|                          | 11.0    | 0.8  | 0.8                        | 0.0   | 11.0               | 0.5                                  | 0.5                        | 0.0  |
|                          | 6.0†    | 11.8   | 0.8                        | 11.0  | 6.0†               | 15.6                                 | 0.5                        | 15.1 |
|                          |         | P <sub>H</sub> 8.2                               |                            |       | P <sub>H</sub> 9.2 |                                      |                            |      |
| Extracellular<br>amylase | 3.0     | 12.1   | 0.9                        | 11.2  | 4.4                | 12.0                                 | 1.7                        | 10.3 |
|                          | 5.6     | 14.2   | 1.0                        | 13.2  | 6.2                | 12.9                                 | 1.7                        | 11.2 |
|                          | 6.0     | 17.2   | 0.8                        | 16.4  | 6.5                | 10.8                                 | 1.7                        | 9.1  |
|                          | 7.0     | 12.6   | 0.8                        | 11.8  | 7.2                | 8.4                                  | 1.7                        | 6.7  |
|                          | 8.0     | 8.4  | 0.9                        | 7.5   | 8.1                | 4.1                                  | 1.7                        | 2.4  |
|                          | 9.0     | 4.1  | 0.9                        | 3.2   | 9.1                | 2.4                                  | 1.7                        | 0.7  |
|                          | 11.0    | 1.0  | 1.0                        | 0.0   | 11.0               | 1.7                                  | 1.7                        | 0.0  |
|                          | 7.2†    | 0.9  | 0.9                        | 9.0   | 7.9†               | 4.6                                  | 1.6                        | 3.0  |
| Intracellular<br>amylase | 3.0     | 12.5   | 1.8                        | 10.7  | 3.0                | 15.8                                 | 3.6                        | 12.2 |
|                          | 5.2     | 14.2   | 1.9                        | 12.3  | 5.4                | 18.4                                 | 3.7                        | 14.7 |
|                          | 5.9     | 14.1   | 1.8                        | 12.3  | 6.0                | 18.6                                 | 3.6                        | 15.0 |
|                          | 6.9     | 11.4   | 1.5                        | 9.9   | 6.9                | 15.8                                 | 3.8                        | 12.0 |
|                          | 8.0     | 11.0   | 1.7                        | 9.3   | 8.0                | 11.3                                 | 3.6                        | 7.7  |
|                          | 9.0     | 7.6  | 1.8                        | 5.8   | 8.4                | 7.1                                  | 3.6                        | 3.5  |
|                          | 11.0    | 2.2  | 1.8                        | 0.4   | 11.0               | 3.6                                  | 3.6                        | 0.0  |
|                          | 6.4†    | 12.4   | 1.8                        | 10.6  | 7.0†               | 17.2                                 | 3.6                        | 13.6 |

\* H-ion concentration of the buffer solution in which enzyme activity was measured.

† Doubly distilled water was substituted for the buffer solution.

$\frac{1}{20}$  of the amylase excreted into the culture medium by one dry weight of the fungus. The amounts obtained with intracellular amylase will represent an average of about  $\frac{1}{3}$  of the total found in the mycelium of one culture.

TABLE V

SHOWING THE ACTIVITY OF INTRA- AND EXTRACELLULAR AMYLASE PRODUCED BY *PENICILLIUM ITALICUM* WHEN GROWN IN CZAPEK'S SOLUTION WITH H-ION CONCENTRATIONS OF  $P_H$  3.0 AND 4.5

| Initial H-ion concentration of Czapek's solution |                       |                   |         |                  |                       |                   |         |                  |
|--|-----------------------|-------------------|---------|------------------|-----------------------|-------------------|---------|------------------|
| $P_H$ 3.0  |                       |                   |         |                  | $P_H$ 4.5             |                   |         |                  |
|  | $P_H$ of buffer sol.* | Cc. N/50 $KMnO_4$ |         |                  | $P_H$ of buffer sol.* | Cc. N/50 $KMnO_4$ |         |                  |
|  |                       | Total             | Control | Amylase activity |                       | Total             | Control | Amylase activity |
|  |                       |                   |         |                  |                       |                   |         |                  |
| Extracellular amylase                            | 3.0                   | 4.5               | 1.5     | 3.0              | 3.0                   | 5.6               | 0.4     | 5.2              |
|  | 5.2                   | 4.8               | 1.2     | 3.6              | 5.4                   | 5.7               | 0.4     | 5.3              |
|  | 6.0                   | 4.9               | 1.5     | 3.4              | 6.0                   | 6.0               | 0.4     | 5.6              |
|  | 7.0                   | 1.7               | 1.3     | 0.4              | 7.1                   | 2.5               | 0.5     | 2.0              |
|  | 8.0                   | 1.5               | 1.5     | 0.0              | 8.0                   | 0.4               | 0.4     | 0.0              |
|  | 9.0                   | 1.5               | 1.5     | 0.0              | 9.0                   | 0.4               | 0.4     | 0.0              |
|  | 11.0                  | 1.5               | 1.4     | 0.0              | 11.0                  | 0.4               | 0.4     | 0.0              |
|  | 5.7†                  | 5.8               | 1.5     | 4.3              | 6.4†                  | 6.4               | 0.4     | 6.0              |
| Intracellular amylase                            | 3.0                   | 15.7              | 3.7     | 12.0             | 3.0                   | 12.7              | 2.5     | 10.2             |
|  | 5.1                   | 16.3              | 3.7     | 12.6             | 5.1                   | 12.2              | 2.4     | 9.8              |
|  | 6.0                   | 16.0              | 3.5     | 12.5             | 6.1                   | 12.5              | 2.4     | 10.1             |
|  | 7.0                   | 9.0               | 3.6     | 5.4              | 7.0                   | 6.7               | 2.5     | 4.2              |
|  | 8.0                   | 3.8               | 3.6     | 0.2              | 8.0                   | 2.5               | 2.5     | 0.0              |
|  | 9.0                   | 3.6               | 3.6     | 0.0              | 9.0                   | 2.5               | 2.5     | 0.0              |
|  | 11.0                  | 3.6               | 3.6     | 0.0              | 11.0                  | 2.5               | 2.5     | 0.0              |
|  | 6.4†                  | 16.6              | 3.6     | 13.0             | 6.4†                  | 10.8              | 2.5     | 8.3              |

\* H-ion concentration of the buffer solution in which enzyme activity was measured.

† Distilled water was substituted for the buffer solution.

It will be seen from the curves (figs. 1-5) and tables II and III, representing the enzyme activity of *Fusarium*, that the effect of the buffer solution of various H-ion concentrations upon the

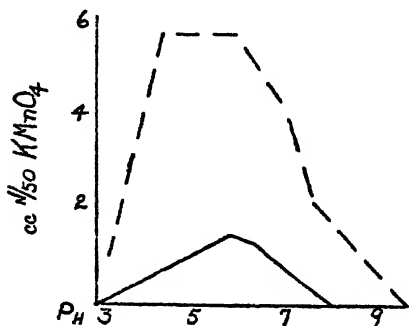


Fig. 3. Action of extracellular (—) and intracellular (---) amylase produced by *Fusarium* sp. grown in Czapek's solution of P<sub>H</sub> 7.0.

extra- and intracellular amylase, produced in culture solutions of the same and of different H-ion concentrations, was similar. The ranges for activity did not correspond in all cases, but this might have been due to the small amount of enzyme material present which at even the optimum H-ion concentration hydrolyzed only a small quantity of starch. It is also likely that under such low enzymic

activity the differences in the original enzyme dispersion, which was the culture solution in one case and an extract of the mycelium in the other, might have been a factor. The amount of starch hydrolyzed at P<sub>H</sub> 3.0 varied, but a maximum was reached at P<sub>H</sub> 6.0 in all of the series. As the buffer solution approached alkalinity, enzyme activity decreased until finally at P<sub>H</sub> 11.0 there was complete inhibition. A rather uniform fall in the activity occurred from P<sub>H</sub> 6.0 to P<sub>H</sub> 11.0.

A similarity will also be noticed for the activity of extra- and intracellular amylase produced by *Colletotrichum* (figs. 6-9 and table IV) at various H-ion concentrations. Inhibition by acid

occurred below P<sub>H</sub> 3.0 and maximum activity was reached at P<sub>H</sub> 6.0, beyond which there was a decrease, and at P<sub>H</sub> 11.0 the enzyme was completely inhibited.

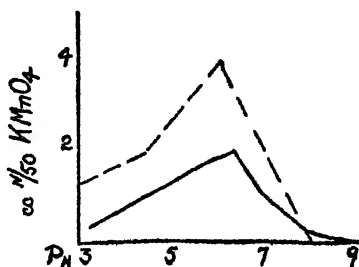


Fig. 4. Action of extracellular (—) and intracellular (---) amylase produced by *Fusarium* sp. grown in Czapek's solution of P<sub>H</sub> 8.2.

From the two series of cultures of *Penicillium* (figs. 10-11 and table v), it is evident that the effect of the buffer solution upon amylolytic activity of the intra and extracellular enzyme in general produced the same results. The range of activity extended from below  $P_H$  3.0 to  $P_H$  8.0 where complete inhibition occurred. The amylase as produced by this organism did not have a sharply defined optimum acidity. Any H-ion concentration between  $P_H$  3.0 and 6.0 seemed to be equally favorable for both the extracellular and intracellular enzyme action.

It is worthy of note that when the activity of amylase in all of the series studied was measured in distilled water instead of the buffer solution (tables II-IV), the results obtained agreed closely with those of the corresponding H-ion concentrations of the buffer solution, thus indicating that at these reactions the ions other than the H ions in the buffer solution had little effect. The distilled water used had an H-ion concentration of  $P_H$  5.4-5.8, but upon the addition of the enzyme dispersion this was shifted toward alkalinity.

When the influences of the H-ion concentration upon the enzyme



Fig. 5. Action of extracellular (—) and intracellular (---) amylase produced by *Fusarium* sp. grown in Czapek's solution of  $P_H$  9.2.

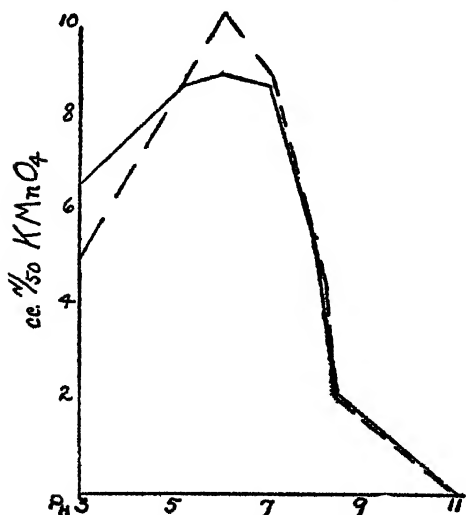


Fig. 6. Action of extracellular (—) and intracellular (---) amylase produced by *Colletotrichum Gossypii* grown in Czapek's solution of  $P_H$  4.5.

secreted by the same fungus grown in nutrient solutions containing varying amounts of acidity and alkalinity are com-

pared, it is evident that the reaction of the medium seemed to have no influence upon the resulting properties as determined by the buffer solution. It must be remembered, however, that the H-ion concentration of the nutrient solution had, in most of the series, been changed during the growth of the fungus. The

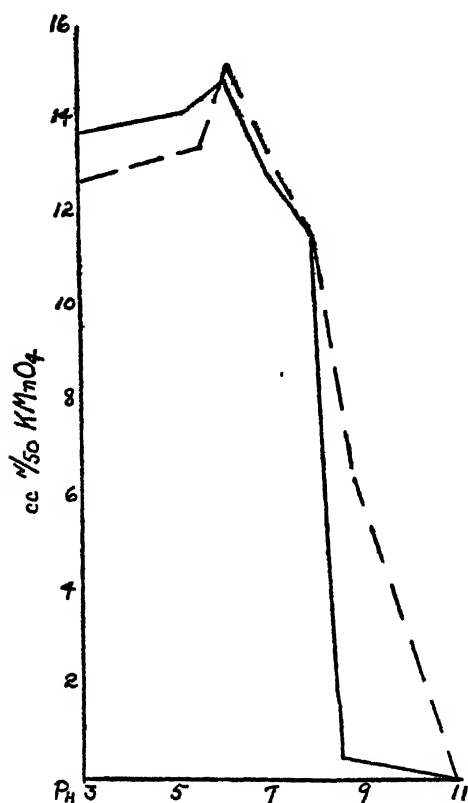


Fig. 7. Action of extracellular (—) and intracellular (---) amylase produced by *Colletotrichum Gossypii* grown in Czapek's solution of  $P_H 7.0$ .

greatest difference, as seen in table 1, was in Series 1 and 5 with *Fusarium* where the final reaction was  $P_H 7.2$  and  $9.2$  respectively. This variation might not have been decided enough to produce a change in the processes within the fungus. The results might have been very different if it had been possible to keep the reaction constant. However, although the  $P_H 9.2$  series of *Fusarium* did not change during the experiment and the more alkaline cultures of *Colletotrichum* were shifted very slightly, the enzyme produced under these conditions was similar to the one produced in the cultures which at the beginning were  $P_H 3.0$  or  $P_H 4.5$ . Further, it is impossible to say what the reaction within the cell

of the fungus has been during the secretion, but it is significant that the enzyme which had been excreted into the culture solution retained the properties of the enzyme in the mycelium. Again, Euler and Emberg ('19) have shown that the enzyme formation by yeast cells could be modified by the adaptation of the

cells to nutrient solutions. If it had been possible to grow the various organisms used in this investigation for several generations upon media having extreme H-ion concentrations with relation to growth more striking results might have been obtained.

Differences with respect to activity range in the buffer solution will be noted in a comparison of the curves of the various organisms. *Fusarium* and *Colletotrichum* resembled each other, while *Penicillium* possessed characteristics somewhat unlike either of these. In the former, maximum activity occurred at  $P_H$  6.0, a gradual decrease followed as the solutions became more alkaline, and complete inhibition occurred at  $P_H$  9.0 or 11.0, depending upon the amount of amylase present in the original enzyme dispersion. In the latter, on the other hand, a zone of maximum action occurred between  $P_H$  3.0, or lower, to  $P_H$  6.0, and activity definitely ceased at  $P_H$  8.0. Even though the enzymes were not purified,

the results would seem to indicate that in *Penicillium* an amylase was formed which had properties somewhat different, at least in regard to activation by the  $H_3PO_4$ -NaOH-starch buffer solution employed for measuring amylase activity.

It was not the purpose of this investigation to establish definite maxima for the amylases produced by these organisms,

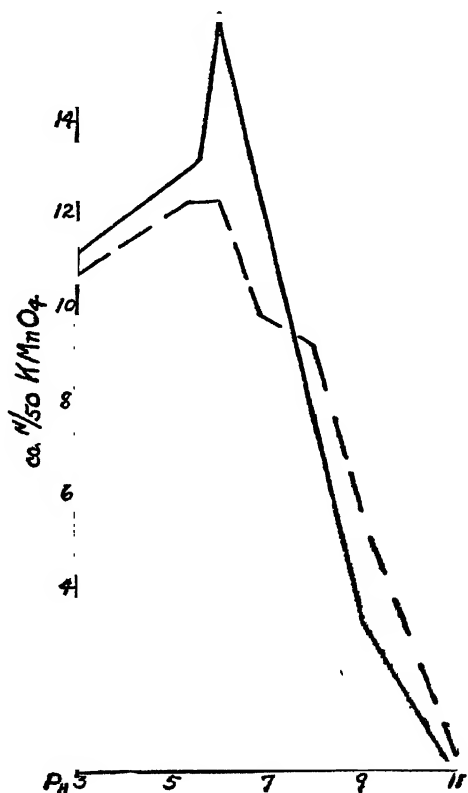


Fig. 8. Action of extracellular (—) and intracellular (---) amylase produced by *Colletotrichum Gossypii* grown in Czapek's solution of  $P_H$  8.2.



still it may be noted that the maximum activity relations as determined by these experiments were different from those obtained by Sherman and his associates ('15-'17) and by Adler ('16). This may be due to the fact that the ions other than the H ions in the solutions used to determine activity exerted an

influence. It is very likely, on the other hand, that the time and temperature of incubation had some influence, since Sørensen showed that the H-ion concentration at which maximum activity was produced, in the case of invertase, depended upon the two above factors and the shift seemed to be toward neutrality with continued incubation.

Since the amounts of the culture solution remaining in the flasks after growth of the fungus were the same in all cases and the dry weights varied very slightly, a summation of the activities of the intra- and extracellular enzymes can be taken to denote relative enzyme accumulation. A relation which varied with the organism seemed to exist between the H-ion con-

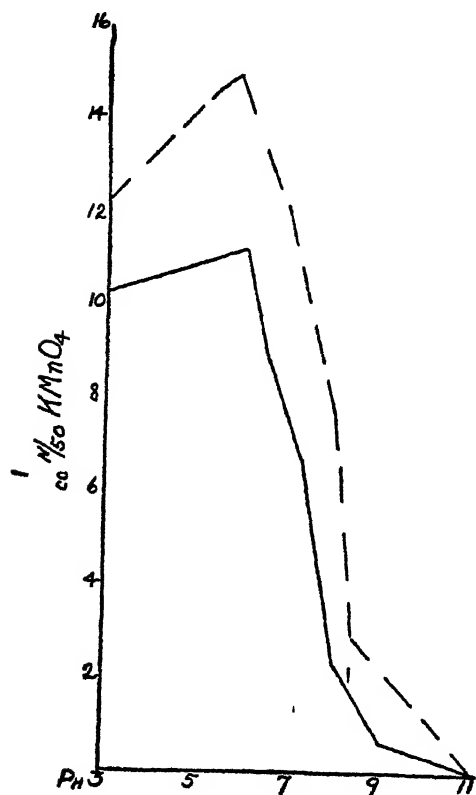


Fig. 9. Action of extracellular (—) and intracellular (---) amylase produced by *Colletotrichum Gossypii* grown in Czapek's solution of P<sub>H</sub> 9.2.

centration of the medium and the accumulation of the enzyme. { If the maximum activity, which is about P<sub>H</sub> 6.0 in the foregoing series, is taken as an index of the amount of amylase present, excretion into the culture solution was greatest for *Fusarium* in natural Czapek's solution, for *Penicillium* in the culture

solution having an initial of  $P_H$  3.0 and for *Colletotrichum* in a medium of  $P_H$  8.2. Intracellular amylase accumulated most abundantly in culture solutions of  $P_H$  3.0 and 8.2 in the cases of *Fusarium* and *Colletotrichum* respectively, while natural Czapek's solution was most beneficial in the case of *Penicillium*.

With *Fusarium*, the greatest amount of total amylase accumulation was in Czapek's solution (fig. 12 and table VI). There was

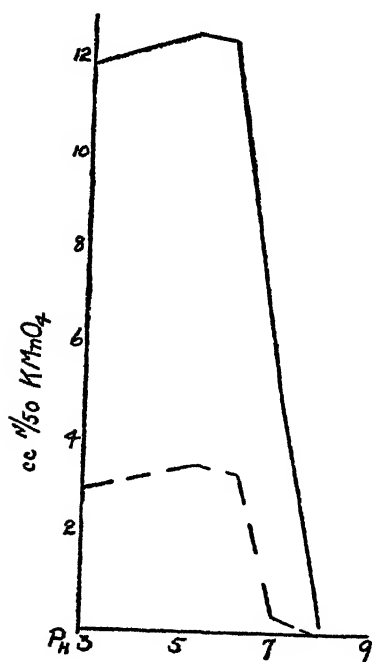


Fig. 10. Action of extracellular (—) and intracellular (---) amylase produced by *Penicillium italicum* grown in Czapek's solution of  $P_H$  3.0.

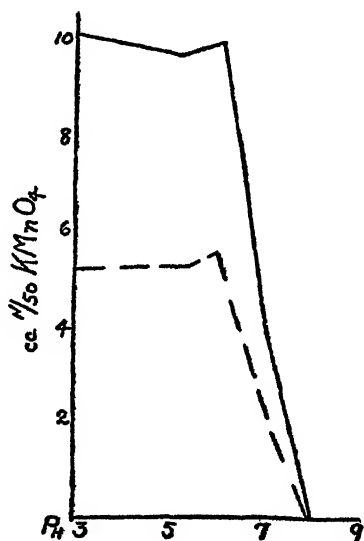


Fig. 11. Action of extracellular (—) and intracellular (---) amylase produced by *Penicillium italicum* grown in Czapek's solution of  $P_H$  4.5.

slightly less in Czapek's solution having an H-ion concentration of  $P_H$  3, and a gradual decrease occurred in Series 3, 4, and 5, respectively. The decrease on the alkaline side may have been due to several factors; either the alkalinity produced less secretion or the amylase was inactivated by these concentrations as it was produced. The amount of mycelium formed does not offer an explanation, since the dry weights of the fungous mats in the case of Series 5 were slightly more than in Series 1. How-

RELATIVE TOTAL ACCUMULATION OF AMYLASE BY *FUSARIUM* SP., *COLLETOTRICHUM GOSYPHII*, AND *PENICILLIUM ITALICUM* WHEN GROWN IN OZAPEK'S SOLUTION OF VARIOUS H-ION CONCENTRATIONS  
(A summation of tables II-IV inclusive)

| Organism                                 | Initial H-ion concentration of Czapek's solution           |  |  |   |  |  |  |  |  |  |
|--|--|--|--|---|--|--|--|--|--|--|
|  | P <sub>H</sub> 3.0   |  | P <sub>H</sub> 4.5   |   | P <sub>H</sub> 7.0   |  | P <sub>H</sub> 8.2   |  | P <sub>H</sub> 9.2   |  |
|  | P <sub>H</sub> of<br>buffer<br>sol.*                       | Cc. N/50<br>KMnO <sub>4</sub> †                  | P <sub>H</sub> of<br>buffer<br>sol.*                       | Cc. N/50<br>KMnO <sub>4</sub> †                   | P <sub>H</sub> of<br>buffer<br>sol.*                               | Cc. N/50<br>KMnO <sub>4</sub> †                    | P <sub>H</sub> of<br>buffer<br>sol.*                       | Cc. N/50<br>KMnO <sub>4</sub> †                    | P <sub>H</sub> of<br>buffer<br>sol.*                                   | Cc. N/50<br>KMnO <sub>4</sub> †                    |
| <i>Fusarium</i><br>sp.                   | 3.0<br>5.3-5.6<br>6.0<br>7.0<br>7.7-8.0<br>8.2-8.6<br>11.0 | 8.2<br>14.5<br>16.9<br>14.6<br>9.8<br>4.0<br>0.4 | 3.0<br>5.2<br>6.0<br>6.9-7.0<br>7.5-8.0<br>8.6-8.7<br>11.0 | 7.0<br>15.7<br>18.7<br>16.9<br>10.5<br>2.8<br>0.0 | 3.0-3.2<br>4.4-5.0<br>6.0-6.8<br>6.9-7.1<br>7.6-8.0<br>9.0<br>11.0 | 1.0<br>6.9<br>7.3<br>5.3<br>2.2<br>0.0<br>0.0      | 3.1-3.2<br>4.4-5.7<br>6.1-6.2<br>7.0<br>8.0<br>9.0<br>11.0 | 1.6<br>3.6<br>3.6<br>3.1<br>0.4<br>0.0<br>0.0      | 3.0<br>5.2-5.5<br>6.0<br>7.0<br>8.0<br>8.3-9.0<br>11.0                 | 2.3<br>5.4<br>5.9<br>4.6<br>3.0<br>1.2<br>0.0      |
| <i>Colletotrichum</i><br><i>Gossypii</i> |  |  | 3.0<br>5.2<br>6.0-6.1<br>7.0<br>8.0-8.2<br>8.4<br>11.0     | 11.6<br>17.6<br>19.3<br>17.8<br>9.9<br>4.2<br>0.0 | 3.0<br>5.1-5.5<br>5.9-6.0<br>7.0<br>8.0<br>8.5-8.8<br>11.0         | 26.6<br>27.8<br>30.0<br>26.3<br>23.2<br>7.2<br>0.0 | 3.0<br>5.2-5.6<br>5.9-6.0<br>6.9-7.0<br>8.0<br>9.0<br>11.0 | 21.9<br>25.5<br>28.7<br>21.7<br>16.8<br>9.0<br>0.4 | 3.0-4.4<br>5.4-6.2<br>6.0-6.5<br>6.9-7.2<br>8.0-8.1<br>8.4-9.1<br>11.0 | 22.5<br>25.9<br>24.1<br>18.7<br>10.1<br>4.2<br>0.0 |
| <i>Penicillium</i><br><i>italicum</i>    | 3.0<br>5.1-5.2<br>6.0<br>7.0<br>8.0<br>9.0<br>11.0         | 15.0<br>16.2<br>15.9<br>5.8<br>0.2<br>0.0<br>0.0 | 3.0<br>5.1-5.4<br>6.0-6.1<br>7.0-7.1<br>8.0<br>9.0<br>11.0 | 15.4<br>15.1<br>15.7<br>6.2<br>0.0<br>0.0<br>0.0  |  |  |  |  |  |  |

ever, this variation was considered within the limits of experimental error, the weights produced being small. It has been shown in these results and also in the investigations of others that alkalinity produces inhibition of enzyme activity. This would explain the small curve obtained for the extracellular amylase. However, a similar reduction occurred for the intracellular enzyme which may mean that either the cell sap had alkaline properties similar to the culture solution and inhibited the enzyme as it was produced, that the culture solutions increased the permeability of the cells to the enzyme, or that the effect was upon the secretion itself.

The results obtained for *Colletotrichum* (fig. 13 and table vi) were quite different from the above. Accumulation seemed to increase as the cultures became less acid, which was evident in both the intra- and extracellular amylase determinations. Maximum accumulation occurred in the  $P_H$  7 series but there was only

slightly less in the  $P_H$  8.2 series. At  $P_H$  9.2 there was more activity than in any one of the series with *Penicillium* or with *Fusarium*. In a comparison of these organisms, it must be remembered that the reaction of the medium after the growth

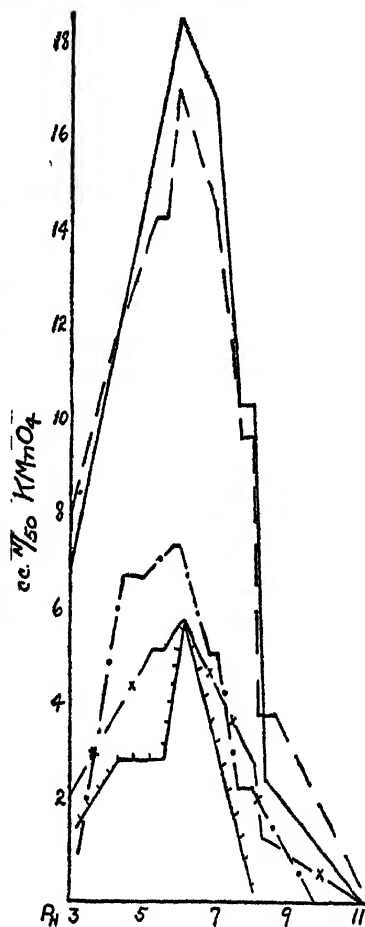


Fig. 12. Total action of extra- and intracellular amylase produced by *Fusarium* sp. grown in Czapek's solution of  $P_H$  3.0 (---),  $P_H$  4.5 (—),  $P_H$  7.0 (-.-),  $P_H$  8.2 (— — —),  $P_H$  9.2 (x—x).

\* H-ion concentration of buffer solution in which enzyme activity was measured.  
† Amounts of sugar formed by extracellular amylase + intracellular amylase, as shown in tables II-IV inclusive.

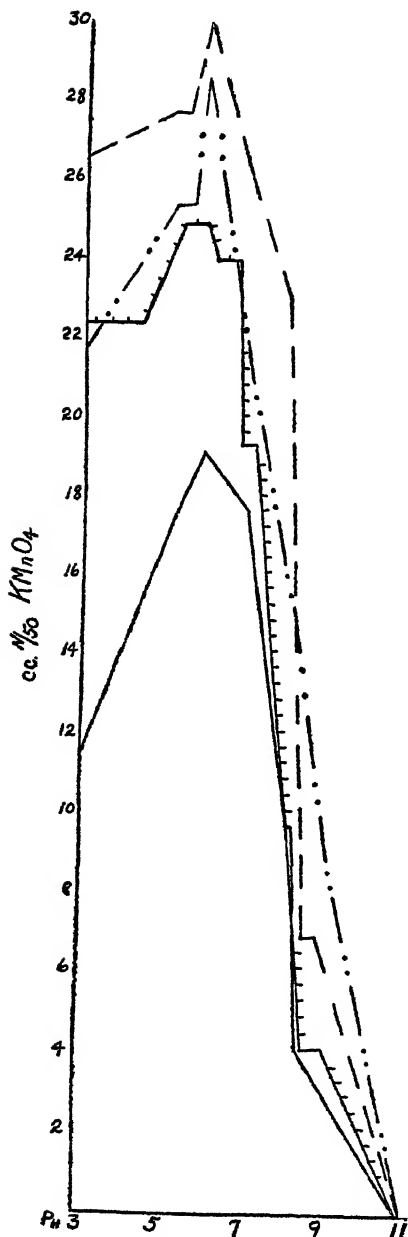


Fig. 13. Total action of extra- and intracellular amylase produced by *Colletotrichum Gossypii* grown in Czapek's solution of  $P_H$  4.5 (—),  $P_H$  7.0 (---),  $P_H$  8.2 (-.-.-),  $P_H$  9.2 (.....).

of the fungus was not the same in the corresponding solutions. Thus for *Colletotrichum* it was  $P_H$  8.4 in Series 8, while under similar initial conditions the final reaction in the case of *Fusarium* was  $P_H$  9.

Sufficient data, in the experiments with *Penicillium*, have not been obtained to establish definite relations. However, natural Czapek's solution and Czapek's solution having a reaction of  $P_H$  3 were equally effective in producing an accumulation of amylase (fig. 14 and table VI). In these determinations, it was evident that the amount of amylase excreted into the culture solution and the amount formed in the mycelium was greater than in the other organisms in the corresponding culture solutions.

A resume of the results with the three fungi showed that maximum amylase accumulation was effected by *Colletotrichum Gossypii* and the least by *Fusarium* sp. Although the results as yet are not inclusive enough to warrant absolute statements, yet they indicate that H-ion concentration of the culture solution may be a factor in amylase secretion. The H-ion concentration at which maximum accumulation was produced varied

with the organism. In *Colletotrichum* it occurred in the cultures having an initial H-ion concentration of  $P_H$  7.0 and a final one of  $P_H$  7.9, and in *Fusarium* in the cultures of  $P_H$  3-4 which finally were  $P_H$  7.2-7.8. The results with *Penicillium* did not cover conditions which were contrasting enough to furnish any conclusions.

### SUMMARY

The activity of amylase produced by fungi grown in Czapek's solution containing starch and having different degrees of acidity and alkalinity has been studied for *Fusarium* sp., *Colletotrichum Gossypii*, and *Penicillium italicum*. The activity of the enzyme as produced under these conditions was measured in NaOH- $H_3PO_4$  buffer solutions having H-ion concentrations from  $P_H$  3.0 to  $P_H$  11.0. An attempt has been made to determine the effect of the reaction of the culture solution upon the accumulation of amylase by the various organisms.

From the results obtained the following conclusions can be drawn:

1. A relation, which varies with the organism, seemed to exist between the H-ion concentration of the medium and the accumulation of extra- and intracellular amylase.

2. In *Fusarium* sp., maximum total accumulation was produced in the solutions having an initial of  $P_H$  4.5 and a final reaction of  $P_H$  7.8, whereas in *Colletotrichum Gossypii* a culture

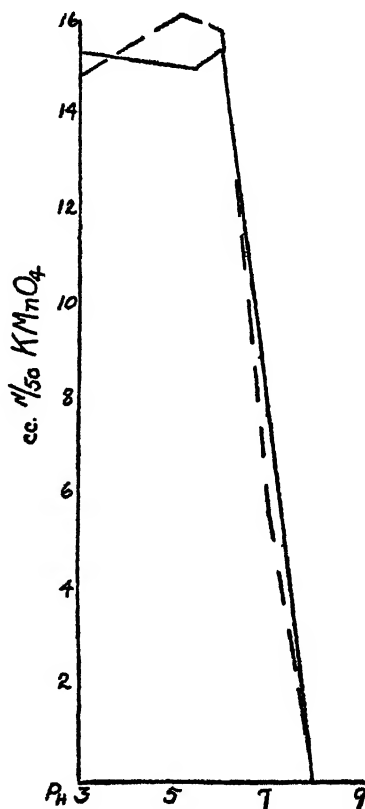


Fig. 14. Total action of extra- and intracellular amylase produced by *Penicillium italicum* grown in Czapek's solution of  $P_H$  3.0 (---) and  $P_H$  4.5 (—).

solution with an initial of  $P_H$  7.0 and a final reaction of 7.9 afforded maximum results, but only slightly less accumulation occurred at  $P_H$  8.2. Culture solutions of  $P_H$  3.0 and  $P_H$  4.5 were equally favorable in the case of *Penicillium italicum*.

3. Amylase accumulated more abundantly in the cultures of *C. Gossypii* than in the other fungi studied.

4. A gradual decrease in the amylase accumulation was effected by *Fusarium* as the culture solution became more alkaline, this decrease not being coincident with a reduction in the amount of growth.

5. An increase in accumulation occurred in the intra- and extracellular amylase of *C. Gossypii* as the nutrient solution became less acid, neutral or alkaline solutions being most effective.

6. The intra- and extracellular amylase, produced by any one fungus under varying H-ion concentrations of the culture solution, had similar properties with respect to the effect of the reaction of the  $\text{NaOH-H}_3\text{PO}_4$  buffer solution upon activation.

7. An optimum zone of activity, between  $P_H$  3.0 and  $P_H$  6.0, existed for *P. italicum*, while in the other fungi the optimum was more sharply defined at  $P_H$  6.0 when the activity was measured in the buffer solution at 28° C. for 24 hours.

8. Complete inactivation occurred at  $P_H$  8.0 for the amylase of *P. italicum*. Under similar amounts of amylase accumulation by *Fusarium* and *C. Gossypii*, inactivation was effected by solutions of  $P_H$  9.0 to 11.0.

9. A decrease in the actual acidity of the culture solution occurred in all of the series of *P. italicum* and all but the most alkaline, or  $P_H$  9.2, series of *Fusarium* and *C. Gossypii*. The former produced no change in the reaction of this culture solution, while the latter caused a slight shift toward neutrality.

Acknowledgements are due Dr. B. M. Duggar, under whose direction the work was carried out, for helpful advice and kindly criticism; and Dr. G. T. Moore for the privileges and facilities of the Missouri Botanical Garden.

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## TWO NEW SENECIOS FROM THE WEST INDIES<sup>1</sup>

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The botanical expeditions to various parts of the West Indies, which have been conducted under the auspices of the New York Botanical Garden during the past twenty years, have materially advanced our knowledge of the flora of that region in securing additional material of many little-known plants and in discovering a considerable number of species new to science. Among recent collections of the genus *Senecio* from the American tropics, which have been submitted to the writer for identification, two from the West Indies appear not to have been previously described. Descriptions of these plants are now placed on record, as follows:

### *Senecio subsquarrosus* Greenman, sp. nov.

Frutex .5-1 m. altus; ramis angulato-canaliculatis dense tomentosis; foliis alternis petiolatis elliptico-oblongis vel subobovatis 5-8 cm. longis 1.5-3 cm. latis obtusis integris vel remote sinuato-dentatis plus minusve revolutisque ad basim cuneatis vel subrotundatis supra tomentulosius primo mox glabratius serius glaberrimis, subtus dense et persistenter tomentosis; petiolis .5-1 cm. longis tomentosis; inflorescentiis terminalibus sessilibus, crebre corymboso-cymosis; capitulis circiter 8 mm. altis discoideis calyculatis; squamellis calyculatis spathulatis 5-7 mm. longis subsquarrosis; involucri squamis 8 erectis linearilanceolatis obtusis 6 mm. longis extrinsecus dense tomentosis;

<sup>1</sup> Issued Jan. 9, 1922.

floribus 10-12; corollis infundibuliformibus flavibus; pappi setis albidis ad corollam subaequantibus; achaeniis hirtellis.

Shrub, .5-1 m. high; branches angulate-channeled, densely tomentose; leaves alternate, petiolate, elliptic-oblong to sub-obovate, 5-8 cm. long, 1.5-3 cm. broad, entire or remotely sinuate-dentate and more or less revolute, tomentulose above in the early stages, but soon glabrate, densely and persistently tomentose beneath; petioles .5-1 cm. long, tomentose; inflorescence a terminal sessile crowded corymbose cyme; heads about 8 mm. high, discoid, calyculate with spatulate 5-7 mm. long subsquarrose bracteoles; bracts of the involucre 8, erect, linear-lanceolate, obtuse, 6 mm. long, densely tomentose on the outer surface; flowers 10-12; corollas tubular-funnelform, yellow; setae of the pappus white, equalling the corolla; achenes hirtellous.—On wet rocks, Rio Guayabo, above the falls, Oriente, Cuba, alt. 450-550 m., 21-30 January, 1910, *J. A. Shafer 3722* (Gray Herb. and N. Y. Bot. Gard. Herb; photograph and fragment in Mo. Bot. Gard. Herb.), TYPE.

The relationship of this species appears to be with *S. carinatus* Greenm. from which it differs in having a crowded and very densely tomentose inflorescence, spatulate subsquarrose bracteoles, eight instead of five involucral bracts, and more numerous flowers in the head.

***Senecio Freemanii* Britton & Greenman, sp. nov.**

Caulis lignescens scandens usque ad 15 m. longus; ramis floriferis teretibus striatis brunneis glabris; foliis alternis petiolatis reflexis ovatis vel ovato-ellipticis 5-8 cm. longis 2.5-4 cm. latis acutis vel obtusis integris utrinque glabris subtus pallidioribus basi in petiolam abrupte contractis, margine plus minusve revolutis; petiolis 6-12 mm. longis; inflorescentiis axillaribus aut terminalibus cymosis parce fulvo-pilosulis paucicapitatis; capitulis 15-18 mm. altis homogamis; involucris anguste campanulatis parce calyculatis; involucri squamis plerumque 8 lineari-lanceolatis acutis 12-15 mm. longis 1.5-3 mm. latis glabris; floribus disci plerumque 18; pappi setis albis; achaeniis circiter 3 mm. longis striato-costatis glabris.

Stem ligneous, scandent, often 15 m. long; flowering branches terete, striate, brownish, glabrous; leaves alternate, petiolate,

reflexed, ovate or ovate-elliptic, 5-8 cm. long, 2.5-4 cm. broad, acute or obtuse, entire, glabrous on both surfaces, somewhat paler beneath, abruptly narrowed at the base into the petiole; margins more or less revolute; petioles 6-12 mm. long; inflorescence axillary or terminal, cymose, sparingly tawny, pilose, few-headed; heads 15-18 mm. high, homogamous; involucre narrowly campanulate, sparingly calyculate; bracts of the involucre usually 8, linear-lanceolate, acute, 12-15 mm. long, 1.5-3 mm. broad, glabrous; disk-flowers usually 18; setae of the pappus white; achenes about 3 mm. long, striate-ribbed, glabrous.—In forest near summit of Mount Tocuche, Trinidad, British West Indies, April 3-5, 1920, *N. L. Britton, T. E. Hazen & Walter Mendelson 1292* (N. Y. Bot. Gard. Herb.; photograph and fragment in Mo. Bot. Gard. Herb.), TYPE.

This species is most nearly related to *Senecio Hollickii* Britton, from which it differs in having larger heads, longer involucral bracts, and glabrous instead of pilose achenes. It is named in honor of MR. W. G. FREEMAN, Director of Agriculture in Trinidad.

## EXPLANATION OF PLATE

## PLATE 1

*Senecio subsquarrosus* Greenman

Cuba

From Shafer No. 3722 in Gray Herbarium  
of Harvard University.



GREENMAN—WEST INDIAN SENECIOS

## EXPLANATION OF PLATE

## PLATE 2

*Senecio Freemanii* Britton & Greenman

Trinidad

From Britton, Hazen & Mendelson No. 1292  
in New York Botanical Garden Herbarium.



GREENMAN—WEST INDIAN SENECIOS





# A MONOGRAPH OF THE GENUS LESQUERELLA<sup>1</sup>

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## GENERAL MORPHOLOGY AND PHYLOGENY

The *Cruciferae* are especially interesting from a phylogenetic point of view because of the importance that attaches to what seem to be minute characters. The greatest students of the subject have failed to find many points of agreement, and there is much confusion in regard to even the broad lines of relationship within the family. It is, of course, a most homogeneous group, and the same variations have occurred independently time and again, thus giving rise to similar forms that are not of necessity analogous. It becomes increasingly evident that before any satisfactory grouping within the family can be made every genus must be studied critically and its connections traced to other genera. It is of fundamental importance to know which species within a group are the primitive ones and which represent terminal branches. When much reliable data of relationship within small groups have been accumulated, and only then, may the parts be finally pieced together, unless perhaps too many intermediates have been irrevocably lost. From present indications, however, an optimistic view may be held with regard to the final solution. In the following treatment of the genus under consideration there have been assembled in paragraphs dealing with particular organs or tissues the various phylogenetic conclusions arrived at and some of the arguments in favor of the different hypotheses. These conclusions, it is believed, point the direction of development in the genus *Lesquerella*, but are not necessarily to be relied upon for any other genus.

*Habit of Growth.*—There are within the genus a few species entirely herbaceous and, under any but the most favorable conditions, certainly annual. Several others have attained an extreme annual condition and pass most of their life within the

<sup>1</sup> Issued Jan. 14, 1922.

protection of the seed. The great majority, however, are definitely perennial and some of these develop a considerable amount of woody tissue in their caudices. The eleven species which constitute the first group are further characterized by branching stems, of which one is formed by the original terminal bud. An extreme development of the annual habit is certainly a definite step in the direction of specialization, although probably a step of no great difficulty. Three species seem to belong to this group of extreme annuals, namely, *L. recurvata*, *L. aurea*, and *L. Palmeri*, and are placed higher in the scale of development than the first group. In these three species the stems are often branched but there is a distinct tendency to an unbranched condition; the terminal bud develops normally. The third category, those species which are definitely perennial, includes some thirty-seven species in varying degrees of development. The extreme development in this group is reached in certain arctic and alpine plants of which *L. arctica* and *L. diversifolia* are examples.

The rosette habit, in which the terminal bud remains undeveloped and lateral stems arise from among the basal leaves, is characteristic of some thirty-three species. In many of these the rosette is strikingly symmetrical and approaches closely to the condition found in certain species of *Physaria*. Some intermediate steps in the formation of rosettes are of particular interest and indicate that the rosette may have arisen in several ways. In a few species, notably *L. Engelmannii*, the terminal bud produces a short, sterile shoot. In *L. lata* and *L. pinetorum* the terminal bud gives rise to a short, fertile shoot that is floriferous nearly or quite to the base, in contrast to the longer lateral stems that bear an inflorescence above their numerous leaves. In *L. intermedia* and *L. arizonica* the terminal bud may give rise either to a normal fertile stem or may be completely inhibited. Branching stems are comparatively rare among the perennial species.

At the top of a list in which the species are arranged in an order of merit based on specialization in the habit of growth is placed *L. Cusickii*. This plant is an annual or short-lived perennial which has evidently been derived from truly perennial

species. In it the rosette is fully developed. Not because of any arguments to be derived from the condition of the roots themselves, have the present phylogenetic conclusions been reached, but because of the correlation with these habits of various other characters that seem clearly to point the direction of the current. The appearance of the rosette habit among the perennials, a character that is clearly derived from the normal condition found in the annual species, is one argument in favor of the present hypothesis that the ancestor of this genus was quite devoid of woody tissue and if not a true annual, was at least entirely herbaceous.

*Shape of the Leaves.*—In the form of the leaves, and particularly of the radical leaves, are to be found some of the most convincing bits of evidence that point the direction in which evolution has occurred. Pinnate, and especially lyrate-pinnatifid, leaves are common throughout the *Cruciferae*, and for this reason alone one would be inclined to consider those species bearing this type of leaf as more primitive than those with entire leaves. It is among the annual species that the pinnate leaf finds its best development, and, indeed, there are none of the purely herbaceous species, except *L. Cusickii*, that do not exhibit this tendency to a more or less marked degree. On the other hand, in only three or four perennial species is the lobing of the radical leaves at all conspicuous. The change from one type to another is very gradual, and the same species may sometimes have entire and sometimes lyrate leaves. Two distinct tendencies are noted: either the leaf may become narrower until a truly linear form is attained, as in the *alpina* group, or it may become broader and the blade abruptly, rather than gradually, narrowed to the petiole. Four species in the genus have cauline leaves that are definitely auriculate at the base and conspicuously toothed. These four species are distributed within the three sections, are all annuals, and all have pinnate basal leaves. Such a condition is believed to represent the ancestral type, and from it to have been derived linear or suborbicular radical leaves and oblanceolate or linear cauline leaves with a narrow base.

*The Flowers.*—In this, as in most other genera of the family, there are few differences in flower parts that offer characters of taxonomic value or help to solve the intricacies of phylogeny.

Because these structures are relatively stable the few differences they do present are often of great value in determining larger circles of relationship and as such are of unusual interest. The sepals are of two nearly equal pairs. They are never truly saccate at the base and are always pubescent with trichomes similar to those borne on other parts of the plant. The petals vary from obovate to narrowly spatulate. They are always entire and there is no distinct differentiation between blade and claw. The broader form is prevalent among the annual species with pinnatifid leaves and presumably represents the original type more closely than the narrow form that is common among the perennial, rosette-forming members of the genus.

The petals are usually yellow and this is undoubtedly to be regarded as the primitive color. In a few species it is pale and variously tinged with red or purple, as in *L. pallida*, *L. purpurea*, and *L. pueblensis*. A more common departure from the normal is found in a number of the more recent forms of widely different groups in which the yellow gives place to a red pigment. This red color may be present only on the tips of the petals as a narrow border or may extend nearly to the base. In some species it apparently appears as the flowers wither. Always its presence or absence is to be regarded as of little significance in the determination of specific limits. This variation has been noted in *L. argyrea*, *L. Fendleri*, *L. Berlandieri*, *L. arenosa*, *L. cinerea*, *L. Kingii* and *L. utahensis* and probably occurs in many others. It is of interest to note here that a similar color change occurs in some species of the related genus *Physaria*.

The staminal filaments present a single character of considerable significance: a conspicuous broadening or dilation at the base, which in its extreme is quite abrupt. Of the six species that show this dilation in a considerable degree, one, *L. Lescurii*, occurs in the section *Alysmus*, one, *L. lasiocarpa*, in *Enantiocarpa*, and four, *L. auriculata*, *L. grandiflora*, *L. densiflora* and *L. angustifolia* in *Eulesquerella*. The four species possessing auriculate cauline leaves, previously considered as most primitive, also possess filaments with dilated bases. The remaining two are primitive in many respects and have certainly not been derived from any more primitive species now existent. All six are annuals and in all six the terminal bud is uninhibited. There

seems no other alternative than to consider this broadened condition of the filament as another character possessed by the ancestral type of the genus.

*The Nectar Glands.*—European botanists have for many years been discussing the taxonomic merits of a study of the nectar glands in the *Cruciferae*, with quite diverse resulting opinions. Much yet remains to be done before their value may be estimated satisfactorily, but it is certain that they must not be neglected in any work, particularly in groups above generic rank, that seeks to unravel natural relationships. Because of the small size and lack of distinguishing color, the difficulty attendant upon their investigation prevents their use in diagnoses or keys intended for popular use and greatly hinders their investigation by the specialist. The degree to which they are developed varies considerably between species and also apparently between individuals, but within the genus the general plan of their arrangement remains fairly constant. These glands consist of elevations of secretive tissue on the receptacle near the bases of the stamens. In *Lesquerella* the greatest development occurs in the immediate vicinity of the base of the solitary stamen and from here extensions reach toward the bases of the double stamens, as shown in the diagrammatic sketch of the location of the glands in *L.*

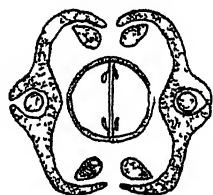


Fig. 1. Diagram showing relative position of nectar glands and stamens in *L. auriculata*.

*auriculata* (fig. 1). Occasionally there is a considerable growth of glandular tissue below or between the double stamens. Sometimes the ring surrounding the solitary stamen may be unbroken on either side or may be open on the opposite side from that shown in the diagram. The glands may be rounded elevations, ridge-like or plate-like masses in different species, or they may be even produced into short, horn-like processes as in *L. lasiocarpa*. In general, the greatest glandular development is to be found in the more primitive species, and probably the glands of the group from which *Lesquerella* was derived were not unlike those of *L. auriculata*.

*The Pedicels.*—Considerable importance attaches to the form of the pedicels in the determination of species because of three characteristic and relatively constant positions assumed. The

first of these is described in the diagnoses as "straight or simply curved upwards" and is to be seen in such species as *L. auriculata*, *L. Engelmannii*, *L. arctica*, *L. globosa*, and *L. Fendleri*. The second type is that known as "recurved." This again is a simple curve and differs from the first in direction and usually in degree: *L. lasiocarpa*, *L. purpurea*, *L. recurvata*, and *L. argentea* serve as illustrations. The third type obtains to some extent in the great majority of species. The curve in this case is a compound one resembling the letter S reversed and lying on its side, and consequently has been termed "sigmoid" or "S-shaped." Species characterized by the third type of pedicel usually have erect pods since the final or distal curve is directed upwards. In general, these three types are easily recognizable but occasionally forms of the sigmoid group are observed in which the final curve has become so nearly obsolete that the pedicel is practically recurved. However, in such cases there is usually at least a suggestion of the final curve in part of the inflorescence and this is enough to indicate the normal condition, since no such tendency is found in any of the species with constantly recurved pedicels. Not a great deal of stress is placed upon the form taken by the pedicel in indicating actual relationships. The first type may be considered primitive since it is characteristic of the family in general and of most of the more primitive species in the genus. The recurved pedicel has probably been developed independently at least three times within the genus, though there is no indication that it has arisen from any except the first type, and from that it is, after all, but a minor change. There is, of course, the possibility that species with recurved pedicels included in sigmoid groups have passed through the stage characteristic of those groups and have subsequently lost the final upward curve—*L. purpurea* and *L. pueblensis* may have had this history. The sigmoid condition is surely a mark of greater specialization than either of the other two, although the change is relatively a simple one. It has probably been derived entirely from the first type and seems to have developed independently at least three and possibly more times. It is noted in such diverse species as *L. Schaueriana* of the section *Enantiocarpa* and *L. argyraea*, *L. alpina*, *L. montana* and *L. Kingii* of *Eulesquerella*.

*The Capsules.*—Of all the various parts of cruciferous plants that are of interest to the taxonomist, the first place is held by the capsules, or pods, since in their component parts occur much greater differentiations than in the more uniform flowers or more readily modified leaves. *Lesquerella* is no exception to this rule, for although we may base theoretical conclusions on the variations assumed by other parts of the plants these conclusions are quite worthless if they do not correlate with those derived from a study of the fruits. Were it not for certain similarities in the structure of the capsules the genus would be incapable of maintenance in its present form and the relationship that is now so evident would become obscure. In order to analyze the various changes that have occurred and to trace their development, the component parts of the fruit will be considered separately and in different paragraphs.

*The Gynophore.*—The gynophore, or stipe that raises the pod above the torus in some species, is a character of considerable importance taxonomically and of great interest from the viewpoint of phylogeny. It is of the more interest because its significance at present is not at all certain. It is present to a noticeable degree in at least five species and reaches its greatest development in the two most primitive of these, *L. Lindheimeri* and *L. gracilis*, in which it is often quite two millimeters long. In the other three, *L. Gordonii*, *L. Garrettii* and *L. latifolia*, though shorter, it is yet quite evident. In some other species, although the stipe is scarcely measurable, the pods are seen to be not truly sessile. *L. Gordonii*, which was undoubtedly derived from *L. gracilis* or a closely related form, is in several ways a step higher in the scale of development and has a reduced stipe. In *L. Palmeri*, which is a western offshoot of *L. Gordonii*, the stipe has disappeared completely. The other two species characterized by the possession of a distinct stipe are not closely related and have their nearest relatives in species with sessile pods. In their cases at least, the stipe is evidently an individual and perhaps an atavistic variation.

Because the most primitive species that show a distinct gynophore have it in its greatest development, and in more recent species it has gradually disappeared, the possibility is suggested that this character was present in the ancestral form of the genus.



This hypothesis seems to be strengthened by the reflection that the stipe is possibly to be considered a primitive character for the family received from Capparidaceous ancestors. The admission of the stipe to the hypothetical ancestor of *Lesquerella* would be very significant and might serve to ally the genus to the *Stanleya-Thelypodium* group, a possibility that should not be forgotten. While it seems well established that the species in which a long stipe is present are to be considered, among living species at least, more primitive than those having a shorter stipe, yet it is also evident that in the most primitive species of the genus the pods are quite sessile. At present, then, we are inclined to believe that the gynophore is a character of no great antiquity in *Lesquerella* and appeared perhaps in a single generation. This view is strengthened by the presence of the stipe in *L. Garrettii* and *L. latifolia* in groups whose ancestors must have had sessile pods and also by the occasional appearance of forms of *L. gracilis* with sessile pods throughout the range of this species.

*The Valves.*—The characteristic form of the pods is largely determined by the size and shape of the two opposing valves. In *Lesquerella* these valves at maturity are dehiscent from the frame-work or replum that bears the placentae and style and across which is stretched the delicate false partition or septum. The valves are rarely, if ever, more than twice as long as wide, are usually more or less inflated or cup-shaped, and are typically without a noticeable midrib. The division into sections is based upon the character of these valves and under the various sections, in a later paragraph, is given the characteristics of each. For the present we will content ourselves with a discussion of the changes that occur in the section *Eulesquerella*, since here are gathered all but four species of the genus. In this section the valves vary considerably between different species by the presence or absence of the stellate trichomes. They vary also in form; some are hemispherical, some lengthened and boat-shaped, some semi-ovoid and one curious form, *L. gracilis* var. *repanda*, bears a shoulder near the base and has an enlarged apex, consequently being obpyriform in outline. Although typically inflated many species possess pods that are variously compressed. Most frequently this compression is at the apex

or on the margins of the septum, and whenever it occurs it is of value to the taxonomist because of its constancy within specific limits. One would scarcely be justified for assuming on any *a priori* grounds that any one form represented the primitive type from which the others have been derived, and it is with great interest that we notice the spherical, glabrous pods of all the species of this section which, because of habit and leaf outline, we have already considered as primitive. In fact, to consider the glabrous, spherical pods as representing the original condition, and the variously elongated and compressed pods that are also almost without exception pubescent, as derived, is quite in harmony with all evidence gained from a study of the variation of parts about whose course of development there can be little doubt.

*The Septum.*—When Watson proposed the genus *Lesquerella* he emphasized the characters of the septum as distinguishing these plants from the Old-World group, *Vesicaria*. Subsequent observation has strengthened the importance of these characters in limiting the genus. Although there is considerable variation between species and even between individuals in the shape of the cells or "areolae" of the septum, there are several points that all the species possess in common. Of first importance, perhaps, is the "nerve," or line that extends from the apex to near the center of the septum. The presence of this nerve is by no means peculiar to *Lesquerella* but serves to separate it from those genera with which it is most likely to be confused. Second, probably, is to be mentioned the attachment of the funiculi to the septum for at least part of their lengths. In some species the attachment is only near the base, while in others the funiculi are attached for over three-fourths their lengths. In all species the attachment is evident upon careful dissection. Here again the character is not peculiar to the present genus but apparently occurs in relatively few genera.

Finally may be mentioned the shape of the cells of the septum. These vary from polygonal to tortuous, but in all the species the boundaries of the cells are distinct and not obscured by numerous lines that would form an anastomosing net-work over the surface. Neither are there present those superimposed fibers characteristic of some groups nor is the effect of numerous par-

allel lines ever produced. The septum is usually entire, but in a number of more highly specialized species is frequently perforate. This character is so variable between species that it seems impossible of use in their delimitation. No attempt has been made to draw phylogenetic conclusions from the characters of the septum, since no definite progression from one species to another has been discovered (fig. 2).

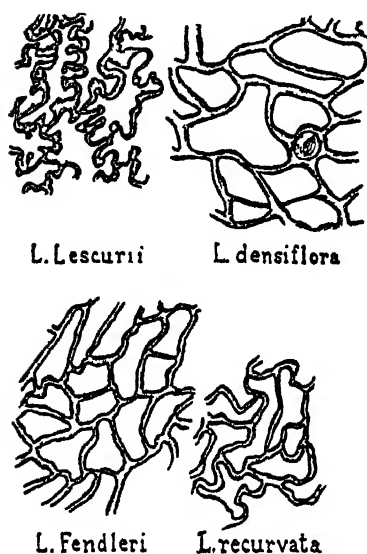


Fig. 2. Characteristic variation of septum between species of *Lesquerella*.

*The Number of Ovules.*—Reduction in the number of ovules within the ovary is so constantly coincident with specialization among dicotyledons in general and in the *Cruciferae* in particular that before examining the species of a given genus one may confidently expect, if any intraspecific variation in the number of ovules occurs, to obtain evidence as to what are the more primitive and what the more recent forms. In the present genus the number of ovules varies between two and sixteen in each cell. While in very few species is the number constant, it varies for a given species within certain limits. It must not be forgotten that the data obtained for the present study were from herbarium material only, and as this was often rather scanty the maxima and minima given are not necessarily exact. It is believed, however, that the relative position of but few species in regard to the number of ovules would be materially changed by increasing the known range of variation. Of particular significance is it to compare pairs of species that are very closely related and of which one has probably been derived from the other. *L. argyraea* and *L. Berlandieri* furnish one interesting case. The former has glabrous pods and the latter stellate-pubescent ones. The former has eight to sixteen ovules and the

latter four to seven. *L. Gordonii* has glabrous pods with four to ten ovules and *L. Palmeri* pubescent pods with four to six ovules. Two species from among the most primitive in the genus in many respects are *L. auriculata* and *L. grandiflora*. The former has a number of long, simple trichomes intermixed with the branching or stellate hairs, has abruptly dilated filament bases, and six to eight ovules; the latter has lost most, if not all, of the simple trichomes, has gradually dilated filament bases, and four to six ovules. A series of three species probably to be placed in linear sequence is that formed by *L. intermedia*, *L. alpina*, and *L. condensata*. The first shows only partial inhibition of the terminal bud, noncompressed pods, and three to eight ovules; the second shows complete inhibition of the terminal bud, pods that are compressed at the apex, and two to four ovules; the third, besides having a terminal bud that remains undeveloped and a pod compressed at the apex, also has greatly reduced stems, and but two ovules. *L. angustifolia* is of particular interest because it has retained so many primitive characters but has only two ovules in each cell.

After considering these facts and many other similar ones it has been felt that above all else the relative number of ovules possessed by any species is indicative of its position in a series. No case is known in which one species, thought because of other characters to have been derived from another, has a greater number of ovules than the supposed parent. A given species may have the largest number of ovules in the genus but on that account has not of necessity the greatest number of primitive characters nor is it to be thought the ancestor of all forms with fewer ovules. All that is indicated is that this species with numerous ovules is more primitive than species closely related to it and having fewer ovules. Furthermore, the species having the most ovules could not have been derived from any other species now extant. *L. argyrea* will serve to illustrate this line of reasoning. It possesses more ovules than any other species of *Eulesgaerella* and is held to be the most primitive species extant of its own particular branch, yet it has lost many characters we believe the ancestral form to have possessed and which are retained by many species with fewer ovules than *L. argyrea*. At every point, conclusions derived from other sources have

been confirmed by a comparative survey of the ovule number in the species concerned.

*The Seeds.*—In this genus the seeds offer little of diagnostic value. Four species have definitely winged or margined seeds. These species are the same that have been previously mentioned as having auriculate stem-leaves and they belong to the three sections. They have already been designated as possessing more primitive characters than any other four species, and consequently this seed character is probably to be considered most primitive also. Throughout the genus the relative position of the cotyledons and the radical is nearly constant and is described by the adjective "accumbent." In this case the radical is applied to the edge rather than to the back of the cotyledons and in cross-section may be diagrammatically represented thus:  $o =$ . In perhaps six species the radical is not centrally placed but twisted slightly to one side and the cotyledon on that side is slightly shorter than on the other. This condition is considered derived, since it occurs in widely diverse and evidently terminal species.

*The Trichomes.*—In the past, attention enough has been paid to the character of the trichomes in cruciferous plants to make it imperative that they be considered critically in any study in any group of species of this family. Many taxonomists have segregated genera largely on the character of the trichomes and some have gone so far as to distinguish tribes by the simple or branched hairs. In *Lesquerella* the species are more or less densely clothed with variously branching hairs which in many cases give them a silvery gray color. Simple trichomes occur regularly in but three species, and in these they are not numerous nor conspicuous and must be searched for with the lens; in a fourth they may be found occasionally. These four species are the same as have been mentioned several times as most primitive and are further characterized by auriculate stem-leaves, winged seeds, and filaments with dilated bases. In these species the branched hairs are frequently not truly stellate by reason of the central ascending axis with branches produced at several heights. Even in the truly stellate hairs the branches are few and always distinct. In the variation of the trichomes in any of the three first-mentioned species, *L. Lescurii*, *L. lasio-*

*carpa*, or *L. auriculata*, may be traced the manner of evolution from a simple hair to a few-rayed, stellate hair. That the simple or few-branched hair is the more primitive form there can scarcely be a doubt, and if there were it would be dispelled by correlating the relative increase in number of rays with the advance of other specializations. In the great majority of species the stellae are nearly symmetrical and the rays are rather conspicuously forked above the base. In the more primitive forms the rays are few and distinct and as progression occurs they become more numerous and are often more or less regularly or irregularly united. Frequently the stellae are conspicuously granular, particularly near the center, with accumulations of lime, but the degree to which these granules are developed and the exact number of the rays vary greatly within the species.

There are two deviations from the normal type of trichome structure. The first of these concerns *L. densiflora* and *L. Engelmannii*, but is more easily observed in the former than in the latter. Its peculiarity consists in a deeper U-shaped notch on one side that renders the stellae somewhat unsymmetrical. This notch is quite universally directed toward the base of the stem or the leaf which would suggest that the stimulus for its development had some connection with gravitational force. Curiously enough even on horizontal stems this notch parallels the axis of the stem and so in this case the notch is at right angles to the pull of gravity. However, this would be explained if these horizontal stems were erect during the period of trichome formation. *L. Engelmannii* possesses this characteristic to a less striking degree, as has already been remarked, and this is due in large part to the more numerous rays which in a measure "crowd out" the notch. These two species, with *L. ovalifolia*, form an interesting series of gradually increasing specialization. In *L. ovalifolia* the stellae are quite symmetrical and the notch has disappeared. The rays are also decidedly more numerous than in *L. Engelmannii*. The second departure from the usual type of stellate trichome unites the six members of the *argyraea* group. Here the rays are described as unbranched, and if they are forked, the forking occurs so near the center of the stars as to render it inconspicuous. The resulting characteristic form is easily distinguished from the more common type by referring to the dia-

grams. The species so segregated are certainly not to be arranged in a linear series but it is believed that they are historically united by some common ancestor. In *L. Schaffneri* of this group the greatest degree of union possible occurs between the rays and a radiately marked scale is produced.

*Summary.*—The more important phylogenetic conclusions reached in the preceding paragraphs may be summed up as follows:

1. The immediate ancestors of this genus are believed to have been entirely herbaceous.

2. Rosette-forming species in which the terminal bud remains comparatively undeveloped are held to have been rather recently derived from species in which there is no inhibition of the terminal bud.

3. From the lyrate-pinnatifid type of radical leaves have been evolved the entire linear or suborbicular leaves.

4. Cauline leaves with auriculate bases are considered more primitive than those which are narrowed to the base.

5. Yellow is the predominating petal color in primitive species, while white, red, or purple is of more recent origin. The obovate petal is more primitive than the narrower forms.

6. The species of *Lesquerella* having abruptly dilated filament-bases are to be considered more primitive in this character than those with gradually dilated or linear filaments.

7. Within the genus there is not a great deal of variation in the nectar glands, but the tendency seems to be toward reduction.

8. The recurved or S-shaped pedicels are thought to have been developed from straight or curved-ascending forms.

9. The length of the gynophore or stipe, when present, seems to decrease with further development, yet it may not have been present in the ancestor of this genus.

10. Glabrous, spherical capsules are considered primitive, at least in the section *Eulesquerella*.

11. The course of evolution has been in every case from the many- to the few-ovuled forms.

12. Seeds provided with a narrow wing or margin are believed to have prevailed among the most primitive species. In a few

recent forms the cotyledons are not quite symmetrical and the radical is slightly turned to one side.

13. Simple or branched trichomes are believed to have given rise to few-rayed and finally to many-rayed stellae.

#### SECTIONAL AND SUBSECTIONAL GROUPS

An endeavor has been made to reconstruct the phylogenetic tree of *Lesquerella* from a knowledge of the tips of the branches with the aid of certain conclusions reached in previous paragraphs as to the trend of evolution (fig. 3). Such reconstructions are necessarily subject to many sources of error, but if they serve no other purpose than to present in a graphic way the author's conclusions, be they correct or not, they are worth while. Only the three main branches have been given definite systematic rank as sections and have been introduced into the taxonomic treatment. The smaller branches have been informally termed "groups" and have been emphasized in no other way than by associating allied species together in the systematic arrangement.

*The Sections.*—It is believed that there were three lines of divergence from some ancient stock. These three primary branches have extant one, three, and forty-eight species respectively, and each branch has been given sectional rank. In habit and majority of vegetative characters the most primitive species of each of these three sections are very similar. Only in the shape of the pods is there any considerable difference. In the first section, *Alysmus*, these are circular and strongly flattened parallel to the septum; in the second, *Enantiocarpa*, the outline of the pods in the most primitive species is also circular but compressed at right angles to the partition; and in the third, *Eulesquerella*, the pods are spherical and in the more ancient species no flattening occurs in either plane. Because from this last, or third, type the two others could have been more easily derived than the second or third type could have been developed from the first, or more easily than the first and third from the second, and because the third type has produced such an immensely greater number of species, it, that is, *Eulesquerella*, is thought to represent the ancient trunk more nearly than do the other two. Members of the first and second sections have been at some



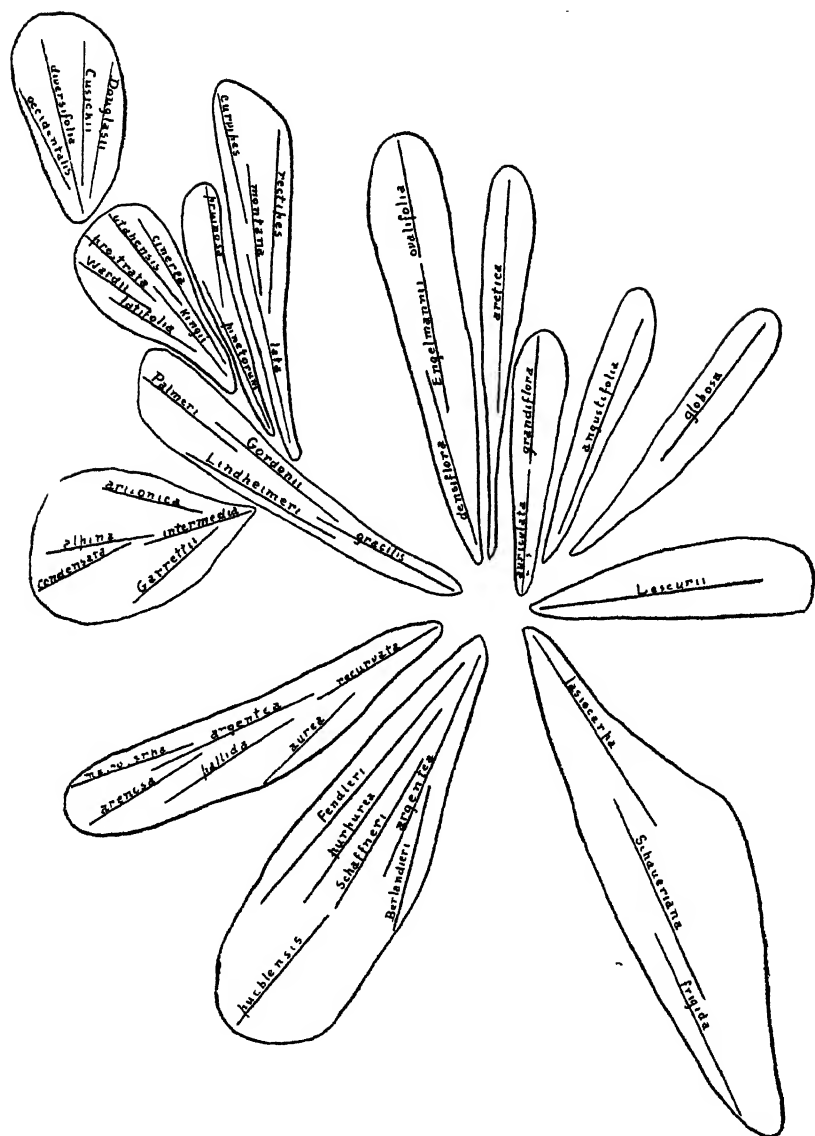


Fig. 3. Phylogenetic chart of the species of *Lesquerella* showing hypothetical relationship of the species. In a general way the chart may be superimposed upon a map of North America with the point of divergence in the Texas region, and a correlation will be seen between the geographical distance of a species from this point and its degree of specialization. In the species of *Euflesquerella* the subsectional groups are indicated by the inclosing lines.

time referred to other genera, but, while they represent natural groups, their interrelationship seems so evident that it appears much the wiser course to unite them under one name.

The first section, as previously stated, contains but a single species, *L. Lescurii*. To the second are referred but three, and one of these, *L. frigida*, has not been seen. The other two, *L. lasiocarpa* and *L. Schaueriana*, although united by the strongly obcompressed pods, show few characters in common. Probably intermediate species may ultimately be reported from the unexplored regions of Mexico. It is the third section, *Eulesquerella*, in which we are particularly interested because here occurs the great mass of species and because many intermediate steps are found between primitive and recent forms.

*The Subsectional Groups.*—The aim has been to bring together into these groups the species that are closely allied. They represent, in a measure, the visible twigs of the phylogenetic tree, although between the units there are frequent gaps of considerable extent. These groups, in some cases at least, may correspond to the "major species" of Hall and Clements.

1. The *auriculata* group contains but two species, *L. auriculata* and *L. grandiflora*, and of these the first is the more primitive. Auriculate stem-leaves, annual roots, dilated filament-bases, and glabrous pods are their chief characteristics.

2. The *Engelmannii* group has been made to include three species, *L. densiflora*, *L. Engelmannii*, and *L. ovalifolia*. The one unusual feature that they possess in common is a contracted and often subumbellate fruiting inflorescence. That the group is a natural one there can be no doubt. Because of the contracted inflorescence the first and second species might seem to be associated, but this could not be certain were it not for the slight asymmetry in the stellae which both exhibit. As has been previously mentioned, this consists in a deeper U-shaped notch on one side of the otherwise symmetrical trichome. Such a peculiarity, it is believed, could scarcely have arisen twice in the same genus. *L. ovalifolia* is a rather recent segregate from *L. Engelmannii* and their affinity is very close, although the U-shaped notch has disappeared in the former. The three species may be placed in linear sequence, each having been derived from the one preceding. Whatever intermediates there might have

been have since disappeared. Besides other advances mentioned elsewhere this group shows a change from sparingly lyrate leaves in *L. densiflora* to entire, oblanceolate leaves in *L. Engelmännii* and finally to entire, ovate leaves in *L. ovalifolia*. Because the pods in this group are not always quite sessile it may possibly have been derived from the *gracilis* group.

3. *L. montevidensis*, although insufficiently known, has an aspect similar to *L. Engelmännii* and may be related to the preceding group.

4. *L. arctica*, with its variety *Purshii*, shows no very close relationship to any other species and is thought to have arisen from some primitive species now unknown. Because of an apparent decrease in the number of ovules and the more frequent appearance of scattered stellae on the pods, the variety is thought to have been derived from the species.

5. The *argyraea* group, previously mentioned as having the rays of the stellae unbranched, contains six species, *L. argyraea*, *L. Berlandieri*, *L. purpurea*, *L. Fendleri*, *L. Schaffneri* and *L. pueblensis*. In this group the second alone possesses pubescent pods but in other ways it is not so specialized as some of the related species. It has probably been directly derived from *L. argyraea*. The other species may have had a common ancestor more primitive even than *L. argyraea*. This was certainly the case with *L. Fendleri*. *L. purpurea* and *L. pueblensis*, although distinguished from the others of this group by their recurved pedicels, are not on this account thought to be closely related. Each of the species in the group stands apart from the others as a distinct unit.

6. The *recurvata* group also includes six species, *L. recurvata*, *L. pallida*, *L. aurea*, *L. argentea*, *L. arenosa*, and *L. macrocarpa*. All these species possess recurved pedicels, stellae with forked rays, and globose pods that are either glabrous or pubescent. *L. pallida* and *L. aurea* seem to represent terminal groups derived from *L. recurvata*. Like it they are annuals. *L. aurea* is of particular interest because the reduction in the number of ovules is extreme and the capsules are either glabrous or stellate-pubescent. *L. argentea*, *L. arenosa*, and *L. macrocarpa* are perennials with densely pubescent pods. *L. argentea*, it is believed, has been derived from *L. recurvata*, although in some respects *L.*

*arenosa* is more similar than *L. argentea* to the primitive form. There can be little doubt that *L. macrocarpa* is a recent offshoot from *L. argentea*. The reduction of its ovules to two confirms this belief. Within this group we see a change from annual to perennial habit occurring associated with a reduction in the number of ovules as well as the appearance of trichomes on the pods. There also occurs the change from sparingly pinnatifid leaves in *L. recurvata* to entire, suborbicular leaves in *L. macrocarpa*.

7. *L. angustifolia*, like *L. arctica*, is a species without close relatives. The glabrous pods and abruptly dilated filament-bases mark it as primitive. The reduction of the number of ovules to two is suggestive of a longer evolutionary history.

8. The *gracilis* group is characterized by the possession of a gynophore in the three primitive species, by sigmoid pedicels in all but *L. gracilis* itself, and by glabrous pods except in *L. Palmeri*. This group is of particular interest on account of the diversity of form between the units involved and because three of the species may be thought to represent three specific generations. *L. Lindheimeri*, although inadequately known, may be regarded as the most primitive of the four species in the group because of the deeply pinnatifid leaves. The other three species, *L. gracilis*, *L. Gordonii*, and *L. Palmeri*, serve to illustrate the shortening of the gynophore as specialization increases. The number of ovules also decreases step by step, and in *L. Palmeri*, in which this reduction reaches the average number of five, the gynophore has disappeared completely and the pods have become pubescent. The variety *repanda* of *gracilis* shows the appearance of a unique character in the genus—the shoulder at the base of the pods. It is not improbable that most of the succeeding groups have developed from this one.

9. The *pinetorum* group consists of but two species, *L. pinetorum* and *L. pruinosa*. They are the only perennials with glabrous pods, sigmoid pedicels, and stellae with branched rays. The first shows only a partial inhibition of the terminal bud, globose pods, and radical leaves that are gradually narrowed at the base. In the second the inhibition of the terminal bud has become complete, the pod elongated, the basal leaves abruptly • narrowed to the petiole, and the number of ovules reduced.

10. The *montana* group, consisting of *L. lata*, *L. rectipes*, *L. montana*, and *L. curvipes*, is, in its most primitive representative, very close to *L. pinetorum* of the preceding group. The only character distinguishing all the species of this group from all of the preceding is the pubescent pods. *L. lata*, like *L. pinetorum*, shows only a partial inhibition of the terminal bud. In all the other species this inhibition is complete. *L. rectipes* and *L. montana* may both have developed from *L. lata*. *L. curvipes* is certainly an offshoot of *L. montana*. The transition from globose to elongated pods with a compressed apex is seen in this group.

11. *L. globosa* is another solitary species derived perhaps from some very ancient form.

12. The affinity of *L. mendocina* remains obscure. Although it has reached a stage of development equivalent to *L. montana*, there is no evidence that they are closely related.

13 The *alpina* group is a homogeneous assemblage of closely related forms characterized by narrow leaves. In the two most primitive of these, *L. intermedia* and *L. arizonica*, the inhibition of the terminal bud varies greatly within the species. *L. alpina* has probably been developed from *L. intermedia* and it in turn has given rise to *L. condensata*. In both these species the inhibition of the terminal bud is complete. The gradual reduction in the number of ovules in the species of this group has been mentioned elsewhere. The appearance of a terminal compression of the pods in *L. alpina* is passed on to *L. condensata*. The origin of *L. Garrettii* is in doubt. Possibly it is an offshoot from *L. intermedia*. *L. valida* has not been seen but seems to be a primitive member of this group. Possibly it is but a form of *L. intermedia*.

14. The *utahensis* group, consisting of *L. cinerea*, *L. Kingii*, *L. latifolia*, *L. Wardii*, *L. utahensis* and *L. prostrata*, is taxonomically the most perplexing group of all. This is due in part to lack of adequate material and in part to a variability of the species concerned. There are within the group no evident lines of development. Rather this seems to represent a plexus of evolution, where the units, be they considered species, races, forms, or varieties of a great polymorphic species, are in a state of change. The group as a whole is characterized by the dense

rosettes, the suborbicular basal leaves, the short stems, and the pubescent, globose, or obcompressed pods. Our interest centers chiefly in *L. utahensis* because it is believed that from it or from some similar form has been developed the genus *Physaria*. Indeed the pods of some individuals of *L. utahensis* suggest that genus almost as much as they do *Lesquerella*.

15. The *occidentalis* group contains the remaining species of the genus, *L. diversifolia*, *L. occidentalis*, *L. Cusickii*, and *L. Douglasii*. The first three of these might be regarded as a single species in a more conservative treatment. They are peculiar because of the compressed margins of the pods. The first named is presumably the most primitive, though not necessarily so. Certainly *L. Cusickii* has been derived from one or the other. *L. Douglasii* does not exhibit the compression of the capsules and has possibly, though not probably, had a separate origin. It, as well as the other three, seems to have been derived from the *utahensis* group.

#### GEOGRAPHICAL DISTRIBUTION

It is believed that in geographical distribution is to be found a most valuable check to any theories of phylogeny derived from purely morphological studies. Furthermore, distribution is an important aid in deciding the relative value of variations for taxonomic purposes. For instance, it is often difficult to decide if a given form merits taxonomic recognition. In the present work the attitude has been taken that if such a form occurs in company with the normal form throughout its area of distribution it is to be regarded as a variation produced by environment, or at least not yet sufficiently distinct to be given a systematic position. If, on the other hand, the variant occurs in a region adjacent to that occupied by the parent species it does certainly deserve treatment by the taxonomist. This attitude is in a measure arbitrary and exceptions have been made when the variation seemed definite enough to warrant it. Besides these reasons for interest in geographical distribution there are certain phases of the subject that deserve consideration on their own account quite apart from any taxonomic or phylogenetic aspect. The preference of many of the species for calcareous soil has been noted, and as a matter of general interest this peculiarity has

been analyzed as far as possible with the data at hand. Certain theories of development depending upon range or area occupied have also been considered in relation to the present genus and evidence concerning their validity obtained. Finally, it has been attempted to procure definite data in regard to the distribution of *Lesquerella* that will serve on comparison with similar data procured from related genera to show any significant differences in the types of distribution.

The species of *Lesquerella* are native chiefly to the arid parts of western North America. Three species occur in South America. One of these, *L. mendocina*, seems to be rather widely distributed across northern Patagonia; another, *L. montevidensis*, has been reported from Uruguay; and the third, *L. frigida*, was collected in the high mountains of Venezuela. In North America three other isolated species are found: *L. arctica* in Greenland and on the shores of arctic America, *L. globosa* in Kentucky and Tennessee, and *L. Lescurii* in the immediate vicinity of Nashville, Tennessee. The remaining forty-six species occur in a nearly continuous area adjacent to the Rocky Mountains from Canada to the southern extremity of the Mexican plateau. On the east this region penetrates to eastern Texas and southwestern Missouri, and on the west to the states that border the Pacific Ocean. The geographical center of this continuous zone lies somewhere in northern New Mexico, but the region of greatest specific concentration is located in central Texas not far from the eastern edge of this area of continuous distribution. Utah and southern New Mexico are also rather remarkable for the number of species that occur within a small area (fig. 4).

*Point of Origin.*—There is much evidence for believing that *Lesquerella* originated at some point in central Texas and from this point as a center has spread over the large area that it now occupies. Other things being equal, one might suppose that migration would take place equally in all directions from the point of origin. It will be seen that the Texas region, although at one edge of the area of continuous distribution, is not far from the center of total distribution. That it is not at the exact center is obviously no argument against the present hypothesis. From purely theoretical standpoints also, the greatest number of species might be expected to occur in the vicinity of the point

of origin, since there the genus would have existed for the longest period of time. In the map showing distribution of species



Fig. 4. Geographical distribution of the species of *Lesquerella* in North America showing relative specific condensation

in North America it is evident at once that the greatest specific concentration occurs in central Texas. It is quite possible, however, that the point of greatest concentration might coincide



with the approximate center of distribution without indicating the point of origin. Evidently much depends upon the character of the species that are here concentrated. Near the place of origin might also be expected the most primitive species. Let us then examine the species found in central Texas. There are ten of these: *L. auriculata*, *L. grandiflora*, *L. densiflora*, *L. Engelmännii*, *L. ovalifolia*, *L. argyrea*, *L. Fendleri*, *L. recurvata*, *L. gracilis*, and *L. Gordonii*. These species, it will be seen, all belong to the section *Eulesquerella* and are those that have been considered among the most primitive in the genus. Six of them are annuals, two have auriculate stem-leaves and filaments with dilated bases, and seven show no inhibition of the terminal bud. Every species of this group has glabrous, spherical pods, and in none is the average number of ovules less than five. Not only are these species primitive, but in no other locality may be found anything like an equal display of what have been considered ancestral characteristics for purely morphological reasons. With central Texas as a center, if one were to draw on the map a series of concentric circles, each succeeding ring would have fewer and fewer species with primitive characters. The periphery in general is bounded by highly specialized members of the genus. In a graphic representation of the subsectional groups they may be shown by lines radiating from a common center. Such a diagram could be superimposed upon a map and in nearly every case the species at the base of each line of development would be nearer the Texas region than species derived from it.

With such an accumulation of evidence pointing to central Texas as the place of origin, for the section *Eulesquerella* at least, and the entire absence of evidence pointing to some other locality as the birthplace of the genus it becomes at least possible to say that if this genus did not originate at this point, there is no evidence to show where it did appear. As for the other sections, it is only possible to say that there is no reason for believing they came into being at a different point. The first consists of a single species and the second of but three so that there is little chance for comparison here. The most primitive member of the second section, *L. lasiocarpa*, occurs in southern Texas and adjacent Mexico; next in order of complexity to it comes *L.*

*Schaueriana* of south central Mexico; and finally the most specialized of the three, *L. frigida*, has been reported from Venezuela. It remains a remarkable probability that from one point should have come so many lines of development.

Six species have been already mentioned as being rather widely isolated from any other representatives of the genus. It is quite worth while to examine these species in detail in order to determine if possible the significance of this isolation. There are at least two possibilities that would account for the location of these species. Either the continuous distribution of the genus may have been much greater formerly than at present and the species that occupied the intermediate areas may have since become extinct, or these isolated forms may owe their location to separate and fortuitous cases of long distance dispersal. If they do represent points in a former continuous distribution, judging from what we know of the other species and their increasing dissimilarity with increased distances from the supposed point of origin, we would expect these far-distant representatives to be the most aberrant and specialized members of the genus. As a matter of fact, the three species occurring in South America and the one in arctic America are very typical members of the genus and find their nearest relatives not in the species closest to them geographically, but in certain ones near the center of distribution. It is of interest also to note that the three South American plants differ considerably from one another and each finds its most nearly related form in North America. Due then to their dissimilarity from one another and their relationship to species near the center rather than on the periphery of the area of continuous distribution, it is thought likely that these four species occupy their present position because of some unknown agent of long-distance dispersal. The two species limited to Kentucky and Tennessee, however, do not seem to fall into the same category with the other isolated forms. Like them they are very dissimilar among themselves, but unlike them they are not similar to species rather near the hypothetical point of origin. Indeed, they are similar to no species now extant and they may well owe their isolation to the extinction of species that once occupied the intermediate areas. The occurrence of colonies of *L. argentea* in localities in Minnesota and Illinois is

curious and is perhaps to be explained by chance dispersal far from the original area occupied by the species.

*Occurrence of the Species upon Calcareous Soils.*—It is evident that many species of this genus occur upon calcareous soils. Pertinent data has been assembled in the hope of observing significant correlations between this habitat and previously obtained phylogenetic conclusions. The following species have been collected upon calcareous soil according to data preserved on herbarium sheets: *L. lasiocarpa*, *L. Schaueriana*, *L. densiflora*, *L. Engelmannii*, *L. ovalifolia*, *L. arctica* var. *Purshii*, *L. recurvata*, *L. gracilis* var. *sessilis*, *L. argyraea*, *L. Fendleri*, and *L. rectipes*. A. Gattinger, in the 'Flora of Tennessee,' notes that *L. Lescurii* occurs only upon calcareous soils. Fernald (*Rhodora* 13: 233. 1911) says that *L. arctica* var. *Purshii* in Newfoundland is a typical calciphile, and Kurtz (Rev. Museo La Plata 5: 286. 1893) observes that *L. mendocina* grows in dry and especially calcareous habitats. Mr. E. J. Palmer has told the author that *L. ovalifolia* seems restricted to limestone regions. Dr. Aven Nelson says that he has collected *L. condensata* upon outcroppings of limestone. Certain other species may be suspected of being calciphiles, since the regions from which they are reported are known to be very largely calcareous. *L. angustifolia* and *L. gracilis* belong to this category. An examination of this list of fifteen species shows a large number of primitive forms. Since the genus is believed to have originated in a region in which limestone is very frequent, this is not surprising. It is of considerable interest to note that primitive species in each of the three sections are calciphiles. Undoubtedly, many species of the genus are not limited to calcareous soils and many more probably never occur on them. Definite data on this point is very meager. *L. montana*, *L. diversifolia*, and *L. Garretii* are known to grow in granitic soils. It will be noticed that these are highly specialized species. Before drawing any conclusions it must be said that from such fragmentary data no more than tentative results can be expected, and since many cruciferous plants are partial to limestone regions this partiality does not necessarily imply close relationship. However, it would seem that the immediate ancestors of *Lesquerella* were calciphytes. A bit of evidence in favor of a common origin

for the three sections is found in the partiality of primitive members of each for soils rich in lime. Similarly, certain widely isolated species are here united to the genus by an additional character.

*Jordan's Law of Isolation.*—It has been found through the study of geographical distribution of *Lesquerella* that in general Jordan's law of distribution holds good. This law is stated in the following terms: "Given any species in any region, the nearest related species is not likely to be found in the same region nor in a remote region, but in a neighboring district separated from the first by a barrier of some sort."<sup>1</sup> In Texas, species that seem not to differ greatly from one another are found in the same region, but practically without exception the species most nearly related is found in an adjacent region. As a rule, the ranges of related species overlap but little and this joint occupation may be apparent rather than real. That the species are separated by a barrier is not always demonstrable. It must be remembered, however, that our knowledge as to what constitutes a barrier is exceedingly limited. A change from calcareous to non-calcareous soil, or a slight difference in water content might form an effective barrier.

*Summary.*—*Lesquerella* seems to have arisen in the central Texas region, and from here the three sections and at least the *auriculata*, *Engelmannii*, *argyraea*, *recurvata*, and *gracilis* groups appear to have diverged. Since most of these various groups have reached a somewhat equivalent degree of specialization and have migrated long distances, it seems probable that they had their origin at about the same period. The Texas species at present appear to be in a rather static condition since the species are easily defined, but in New Mexico and Utah occur recent plexes of development to judge from the variability and number of the closely related species. From the Utah plexus it is believed that the genus *Physaria* has arisen. The primitive representatives of *Lesquerella* were probably partial to calcareous habitats. Since Jordan's law of isolation seems to be supported by the present study, it will be necessary to take into account the characteristic separation of specific ranges in any inquiry

<sup>1</sup>Jordan, D. S. The origin of species through isolation. *Science* N. S. 22: 545. 1905.

that seeks to explain the cause or mechanism of species formation in *Lesquerella*.

#### TAXONOMIC HISTORY OF LESQUERELLA

*Position of Lesquerella among Other Genera of the Cruciferae.*—There have been three rather recent treatments of the American genera of this family in which an endeavor was made to observe phylogenetic sequence. Prantl in 'Die Naturlichen Pflanzenfamilien' united six genera of more or less similar aspect into the subtribe *Physariinae* of the tribe *Schizopetaleae*. These genera are *Synthlipsis*, *Lyrocarpa*, *Dithyrea*, *Physaria*, *Lesquerella*, and *Phoenicaulis*. Robinson in Gray's 'Synoptical Flora of North America' retained this group with the exception of *Phoenicaulis*, under the tribal name of *Physarieae*. Von Hayek, in the most recent review of the family as a whole (Beih. Bot. Centralbl. 27: 310. 1911), preserves the designations and ranks proposed by Prantl, but transfers *Synthlipsis* to another subtribe of the *Schizopetaleae* and adds *Mancoa*, *Agallis*, *Sphaerocardamum*, *Stenonema* and *Notothlapsi* to the subtribe *Physariinae*. For *Lyrocarpa* he erects a separate subtribe. In a diagrammatic representation of the phylogeny of the tribe he derives *Lesquerella* from *Mancoa* and from *Lesquerella* he derives *Physaria*. This latter supposition is confirmed by the present study. The reason for relating *Lesquerella* to *Mancoa* is not evident. This latter genus is represented by a single annual species in the Andes of Peru and Argentina. It is further characterized by elongated, somewhat obcompressed, pubescent pods, and white flowers. Because of the obcompressed pods of the species in the section *Enanthocarpa* they have been referred by some authors to the genus *Synthlipsis*. As stated in another part of the present study, it is not believed that these species are at all related to the type of *Synthlipsis*. In fact, *Physaria* is the only genus that may with confidence be associated with *Lesquerella* at the present time. In any search for the group from which *Lesquerella* has been evolved the most primitive characters must be constantly borne in mind as well as the probable point of origin. What the ancestral genus was we do not yet presume to say.

*Previous Taxonomic Treatments of the Species of Lesquerella.*—The first species of this genus to be definitely recorded by science

were *L. arctica* and *L. globosa* in 1814. The first of these was described as a species of *Alyssum* by Hornemann and the second as a *Vesicaria* by Desvaux. Two years later Pursh described a third species as *Myagrum argenteum*. For nearly seventy-five years after the first species was described, the new species of this genus that were reported from time to time were almost without exception assigned to the genus *Vesicaria*. The first treatment of these American plants that pretended to be anything more than a mere compilation was published by Dr. Gray in 1850 in the Boston Journal of Natural History. This synopsis included nineteen species and was the outgrowth of the study made by Dr. Gray of the collections of F. Lindheimer in Texas. The second notable contribution to a knowledge of this group was published by Sereno Watson in the Proceedings of the American Academy of Arts and Sciences for 1888. Here Watson recognized thirty-three species and called attention to their generic dissimilarity to the Old World members of the genus *Vesicaria*. He proposed the name *Lesquerella* for the American forms "in honor of our venerable and in every way worthy paleontologist and bryologist, Leo Lesquereux." This revision remains essentially unchanged to-day except for the introduction of a number of species unknown to Watson and the incorporation of a definite theory of phylogeny. Subsequent to the publication of the 'Synoptical Flora of North America' in 1895, that contained a recapitulation of Watson's earlier work, no treatment of the genus as a whole has been attempted. The validity of *Lesquerella* as a generic concept has rarely, if ever, been questioned in America, and all recent students of the *Cruciferae* who have published on this group have realized that the American plants are not closely related to the European and Asiatic plants. The resemblance, although sometimes striking, is evidently superficial. Aside from the earlier taxonomic works on *Lesquerella*, we are greatly indebted to many recent treatments of the species of particular regions that have been published in various "Manuals" or "Floras."

*The Type Species of Lesquerella.*—It is becoming increasingly evident that taxonomists must base their generic concepts definitely on a certain species just as they base their specific concepts on a particular specimen. It is recognized that there

are within the genus, as here interpreted, three fairly distinct sections that from a narrower point of view might be considered separate genera. It is obviously necessary to locate that portion of the group that would retain the name *Lesquerella* were the sections elevated to generic rank. We must decide, if possible, upon a type species for the genus. Unfortunately *L. Lescurii* (Britton & Brown, Ill. Fl., ed. 2, 2: 154. 1913) has been previously designated as the type species because it occupies first place in the original publication of *Lesquerella*. This was apparently done without due regard to the very reasons that placed it first in the list of species. The International Rules of Botanical Nomenclature adopted at Vienna in 1905 are very clear as regards that portion of a genus that is to retain the original name. Article 45 reads in part: "When a genus is divided into two or more genera, the name must be kept and given to one of the principal divisions. If a genus contains a section or some other division, which, judging by its name or its species, is the type or origin of the group, the name is reserved for that part of it." The example for this point is taken from the genus *Helianthemum*. The genus contained originally nine sections; "several of these sections have since been raised to generic rank but the name *Helianthemum* has been kept for the divisions grouped round the section *Euhelianthemum*." In the original description Watson divided the genus into the sections "*Alysmus*" and "*Lesquerella*, proper." Probably because he regarded the first group as the more primitive as well as somewhat aberrant, he placed it first. The most aberrant species of all he placed first in the section. Of this species he said: "Our one flat-podded species that has been referred to *Alyssum* (*A. Lescurii*) appears to differ in no other respect than its less convex valves from a somewhat distinct group of species which can be separated, however, only as a section from the rest." To accept this species as the type species of the genus would certainly be contrary to what the author had in mind when he described the genus. If the genus were divided, a step we would greatly deplore, the International Rules point definitely to the section "*Lesquerella* proper," or "*Eulesquerella*," as the part that would retain the present generic name. From this section as outlined by Watson the type species

must certainly be selected. Watson lists *L. occidentalis* first under this group, and since the relationship of this species is clearly with the great mass of species attributed to this genus it is here designated as the type species of *Lesquerella*.

*Acknowledgments.*—The student of taxonomy is indebted to so many sources for his material that it is quite impossible to recognize even some of the most important in a formal manner. Personal gratitude may not be the criterion. The reader has, however, some rights in the matter and should know some of the sources of the material with which the author worked and the facilities at his disposal. The present study was made at the Missouri Botanical Garden, thanks to the generosity of the Director, Dr. George T. Moore, and under the constant supervision of Dr. J. M. Greenman, Curator of the Herbarium. Besides the collections in the Garden Herbarium specimens were borrowed from the United States National Herbarium, the Gray Herbarium, the Rocky Mountain Herbarium, the herbarium of Mr. George E. Osterhout of Windsor, Colorado, the herbarium of Prof. E. Bethel of Denver, Colorado, the Baker Herbarium at Pomona College, the herbarium of Mr. C. C. Deam, of Bluffton, Indiana, and the herbarium of Mr. I. W. Clokey, of Denver, Colorado. To the owners and curators of these various collections the author is greatly indebted.

*Illustrations.*—The illustrations were made by the author from herbarium material. The drawings of the trichomes were made with the aid of the camera lucida.

#### TAXONOMY

*LESQUERELLA* Watson, Proc. Am. Acad. 23: 249. 1888; Prantl in Engler & Prantl, Nat. Pflanzenfam. III. Abt. 2: 187. 1891; Coulter, Contr. U. S. Nat. Herb. 2: 17. 1891; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 116. 1895; Britton & Brown, Ill. Fl. 2: 136. 1897, ed. 2, 2: 154. 1913; Heller, Cat. N. Am. Pl. 88. 1900; Small, Fl. Southeastern U. S. 468. 1903, ed. 2, 468. 1913; Rydb. Fl. Colo. 154. 1906; Robinson & Fernald in Gray, Manual, ed. 7, 424. 1908; Coulter & Nelson, Manual Cent. Rocky Mountains, 218. 1909; Degen, Magyar Bot. Lap. 8: 3. 1909; Hayek, Beih. Bot. Centralbl. 27: 310. 1911; Wootton & Standley, Contr. U. S. Nat. Herb. 19: 274. 1915; Rydb. Fl. Rocky Mountains, 331. 1917.



*Vesicaria*, section *Vesicaria* DC. Syst. 2: 295. 1821, in part.  
*Vesicaria*, section *Vesicariana* DC. Prodr. 1: 159. 1824, in part; Walp. Rep. 1: 140. 1842, in part; Dietr. Syn. Pl. 3: 638. 1843, in part.

*Vesicaria* Torr. & Gray, Fl. N. Am. 1: 100. 1838; Gray, Gen. Am. Bor.-Or. Ill. 1: 161. 1848; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 148. 1850; Walp. Ann. 2: 37. 1851; Benth. & Hook. Gen. Pl. 1: 73. 1862, in part; Wats. Bibliog. Ind. N. Am. Bot. 74. 1878; Coulter, Manual Rocky Mountain Region, 25. 1885.

*Alyssum* Gray, Manual, ed. 5, 72. 1867, in part; Ktze. Rev. Gen. Pl. 2: 931. 1891.

*Synthlipsis* Wats. Bibliog. Ind. N. Am. Bot. 72. 1878, in part; Coulter, Contr. U. S. Nat. Herb. 2: 21. 1891, in part; Wats. in Gray, Syn. Fl. N. Am. 1: 121. 1895, in part; Small, Fl. Southeastern U. S. 468. 1903, ed. 2, 468. 1913.

*Alyssum*, section *Vesicariana* Ktze. in Post & Ktze. Lexicon Gen. Plant. 21. 1904, in part.

Annual, biennial, or perennial herbs more or less densely covered with branching or stellate hairs. Stems simple or branched, both terminal and lateral developing, or frequently, due to the more or less complete inhibition of the terminal bud, only the lateral produced from a basal rosette. Radical leaves from deeply pinnatifid and thin to oblanceolate and entire or even suborbicular and then usually thick. Cauline leaves in a few species auriculate at the base, but usually oblanceolate and subentire with a slender, cuneate petiole. Flowers sometimes large and showy, frequently rather small and inconspicuous; petals usually yellow, occasionally red or purple and rarely nearly white, entire, obovate to narrowly spatulate; filaments linear, edentate, in a few species dilated at the base. Pedicels straight, simply curved upwards, recurved or sigmoid. Pods sessile or stipitate, glabrous or stellate-pubescent, typically spherical, often flattened at the apex, sometimes elongated but rarely more than twice as long as wide; in one section the pods are strongly flattened parallel to the septum, in another at right angles to it. Styles persistent, usually long and slender. Stigmas capitate, slightly two-lobed or scarcely enlarged. Septum usually thin, nerved from the apex towards the base; areolae

from polygonal to tortuous; superimposed fibers wanting. Ovules 2-15 in each cell, funiculi attached to the septum for part of their lengths. Seeds more or less flattened, winged or immarginate; cotyledons accumbent, radical sometimes slightly turned to one side.

### KEY TO THE SECTIONS

- Pods strongly flattened parallel to the septum, hirsute; stem-leaves auriculate..... §1. *Alysmus*  
 Pods various, if hirsute not flattened parallel to the septum; stem-leaves various.  
 Valves compressed at right angles to the septum, pubescent.. §2. *Enantiocarpa*  
 Valves various but never compressed at right angles to the septum..... §3. *Eulesquerella*

### SECTION 1. ALYSMUS Wats.

§ 1. *ALYSMUS* Wats. Proc. Am. Acad. 23: 250. 1888; Prantl in Engler & Prantl, Nat. Pflanzenfam. III. Abt. 2: 188. 1891; Wats. Syn. Fl. N. Am. 1': 116. 1895.

Annual, sparsely stellate; terminal bud developing into main stem; stems branching; radical leaves lyrate-pinnatifid, cauline leaves auriculate at the base; filaments dilated at the base; pods strongly flattened parallel to the septum, orbicular, sessile, sparingly stellate and ciliate; ovules 3-5 in each cell, funiculi free or attached to septum only at the base; seeds flat, narrowly winged. Species 1.

1. *Lesquerella Lescurii* (Gray) Wats. Proc. Am. Acad. 23: 250. 1888; Wats. Syn. Fl. N. Am. 1': 116. 1895; Chapman, Fl. Southern U. S. 29. 1897; Britton & Brown, Ill. Fl. 2: 154. 1913; Small, Fl. Southeastern U. S. 469. 1903, and ed. 2, 1913.

*Vesicaria Lescurii* Gray, Manual, ed. 2, 38. 1857; Torr. & Gray, Fl. N. Am. 1: 100. 1838.

*Alyssum Lescurii* Gray, Manual, ed. 5, 72. 1867.

Annual or biennial, sparsely stellate-pubescent, with few-rayed, rather loose stellae; stem repeatedly branching, particularly near the base, 1-3 dm. long; branches slender, spreading or ascending; terminal bud developing into main stem; radical leaves withering early, 4-10 cm. long, lyrate-pinnatifid, with rather few, remote segments, tapering to a slender petiole; cauline leaves thin, shallowly toothed, oblanceolate to ovate or narrower, 1-3 cm. long, auriculate at base; flowers yellow;

petals about 4 mm. long, broadly unguiculate, not enlarged at the base; filaments dilated at base; fruiting inflorescence elongated; pedicels ascending-divergent, about twice as long as the pods; pods erect, sessile, orbicular or slightly longer than broad, 3-5 mm. in diameter, strongly flattened parallel to the partition, valves slightly arched, hirsute with simple or sparingly branched hairs having conspicuously enlarged bases, small, stellate hairs sparingly intermixed; short midvein evident at base of valves; styles 1-2 mm. long; stigmas capitate; septum dense, nerved from apex over half way to base, areolae tortuous; ovules 3-5 in each cell, funiculi attached to septum only at the base; seeds flat, narrowly winged.

Distribution: in the vicinity of Nashville, Tennessee.

Specimens examined:

Tennessee: Nashville, 1855, *Lesquereux* (Mo. Bot. Gard. Herb.); "raised from seed sent me from Edgefield Junction," 1871, *Porter* (Mo. Bot. Gard. Herb.); hills around Nashville, April and May, 1879, *Gattinger* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Nashville, May, 1879, *Gattinger* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); near Nashville, June, 1880, *Hubbard* 185 (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Nashville, 1896, *Barnes*, (U. S. Nat. Herb.); west Nashville, May 26-27, 1909, *Eggleston* 4419 (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

A species of restricted range more or less anomalous in *Lesquerella* because of the strongly flattened pods and the septum which is quite different from that in most other species of the genus. Characters shown by this species—for example, auriculate stem-leaves, dilated filament-bases, and winged seeds—indicate its relationship to the more primitive species of the other sections, although they are not found in the majority of the species of the genus. It exhibits the characteristic median nerve of the septum found throughout the group. The dense septum is not greatly unlike that of some representatives of the section *Eulesquerella*. There remains, then, no character to keep this plant out of the genus *Lesquerella* except the pod flattened parallel to the septum, and it were much better to retain it with its relatives than to erect a monotypic genus on this character alone.

The limited range of this species is interesting because in the vicinity of Nashville, the only place it is known to occur, it is reported as being abundant. One wonders what the limiting factor in its distribution may be.

## SECTION 2. ENANTIOCARPA Payson

### § 2. ENANTIOCARPA Payson, new section.

Annuals or perennials; terminal bud usually developing into a fertile stem; radical leaves entire to lyrate; cauline leaves auriculate at the base and sessile or narrowed to a petiole; filaments gradually dilated at the base or linear; pods strongly compressed at right angles to the partition; seeds winged or immarginate. Species 2-4.

### KEY TO THE SPECIES

Leaves more or less toothed or pinnatifid.

Pods nearly orbicular, pendent.....2. *L. lasiocarpa*

Pods elliptical, erect on sigmoid pedicels.....3. *L. Schaueriana*

Leaves quite entire.....4. *L. frigida*

2. *L. lasiocarpa* (Hook.) Wats. Proc. Am. Acad. 23: 251. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 116. 1895; Small, Fl. Southeastern U. S. 469. 1903, and ed. 2, 469. 1913; Payson, Ann. Mo. Bot. Gard. 5: 143. 1918.

*Vesicaria lasiocarpa* Hook. (name only) Bot. Mag. N. S. 10: under t. 3464. 1836; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 150. 1850 (name only); Gray, Smithson. Contr. (Pl. Wright.) 5: 13. 1853 (description).

*Synthlipsis Berlandieri* Gray var. *hispida* Wats. Proc. Am. Acad. 17: 321. 1882; Coulter, Contr. U. S. Nat. Herb. 2: 21. 1891; Heller, Contr. Herb. Franklin and Marshall College 1: 40. 1895.

*S. heterochroma* Wats. Proc. Am. Acad. 17: 321. 1882.

*Alyssum lasiocarpum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Annual or biennial; somewhat canescent, with small, irregularly branching or stellate hairs and more or less hirsute with simple trichomes; stems decumbent or procumbent, 1-6 dm. long, in the larger plants usually branched; terminal bud of rosette usually developing; radical leaves thin, soon withering, often 1 dm. long, oblanceolate in outline, irregularly and deeply lyrate, obtuse, scarcely petioled; cauline leaves 1-6 cm. long, oblanceo-

late or obovate, often incisely pinnatifid, always conspicuously toothed, often more or less auriculate at the base; flowers at

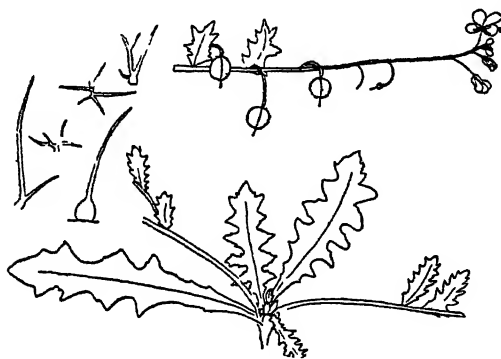


Fig. 5. *L. lasiocarpa*. Habit sketch  $\times \frac{1}{8}$ . Trichomes  $\times 25$ .

first yellow, apparently turning purplish on withering; petals 6–10 mm. long, broad; filaments gradually dilated at the base; fruiting inflorescence elongated, open; pedicels slender, about 2 cm. long, recurved; pods pendent, sessile, decidedly flattened contrary to the septum, circular or obovate, 7–9 mm.

long, with small, stellately branching hairs intermixed with simple trichomes often enlarged at the base, young pods conspicuously hirsute; styles about 2 mm. long; stigmas conspicuous; septum membranous, nerved from apex toward the base, areolae somewhat tortuous; ovules 10–15 in each cell, funiculi long and slender, attached to septum at base; seeds flat, narrowly margined.

Distribution: southern Texas and northeastern Mexico.

Specimens examined:

Texas: railroads near Victoria, Victoria County, April 7, 1900, *Eggert* (Mo. Bot. Gard. Herb.); Corpus Christi, March 5–12, 1894, *Heller 1405*, in part (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); shell marl banks along beach, Corpus Christi, March 8, 1917, *Palmer 11215* (Mo. Bot. Gard. Herb.); open ground, Alice, Jim Wells County, March 13, 1917, *Palmer 11259* (Mo. Bot. Gard. Herb.); Eagle Pass, May, 1883, *Havard* (U. S. Nat. Herb.); sands, Laredo, March 20, 1903, *Reverchon 3719* (Mo. Bot. Gard. Herb.); sandy banks of Rio Grande, Webb County, April 9, 1901, *Eggert* (Mo. Bot. Gard. Herb.); Brazos Santiagos, 1889, *Nealley 147* (U. S. Nat. Herb.).

Mexico:

Nuevo Leon: Feb.–Oct., 1880, *Palmer 33* (U. S. Nat. Herb.); Monterey, March, 1891, *Dodge 51* (U. S. Nat. Herb.); Hacienda

El Carrizo, Feb. 28, 1906, *Pringle 10236* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Tamaulipas: vicinity of Victoria, Feb. 1–April 9, 1907, *Palmer 41* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.).

Vera Cruz: vicinity of Pueblo Viejo, 2 kilometers south of Tampico, Feb. 10–25, 1910, *Palmer 366* (Mo. Bot. Gard. Herb.).

2a. Var. *Berlandieri* (Gray) Payson, new comb.

*Synthlipsis Berlandieri* Gray, Bot. Mex. Bound. Surv. 34. 1859; Small, Fl. Southeastern U. S. 468. 1903, ed. 2, 468. 1913.

This differs from the species in being less hirsute throughout and in having no simple trichomes whatever on the pods.

Distribution: From Corpus Christi, Texas, to Matamoros, Mexico.

Specimens examined:

Texas: Corpus Christi, March 5–12, 1894, *Heller 1405*, in part (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Corpus Christi, March 31, 1905, *Tracy 9348* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb., in part).

Mexico:

Tamaulipas: Matamoros, Feb., 1832, *Berlandier 3102* (Mo. Bot. Gard. Herb.); Matamoros, *Berlandier 3017* (U. S. Nat. Herb.).

*L. lasiocarpa* and its variety have been maintained in herbaria and literature for many years under two generic names, *Lesquerella* and *Synthlipsis*. Since its pods are flattened contrary to the narrow partition it has been associated with *Synthlipsis Greggii* from which in other respects it is quite different. It has, indeed, no characters to keep it out of *Lesquerella* and to the species of that genus it shows many points of similarity. In the author's opinion this species is to be considered rather near the great plexus of the genus from which arose the three sections. This view explains the many points of similarity to certain species placed in other sections.

3. *L. Schaueriana* (Kuntze) Payson, new comb.

*Vesicaria argentea* Schauer, *Linnaea* 20: 720. 1847; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 150. 1850.

• *Lesquerella ?argentea* Wats. Proc. Am. Acad. 23: 252. 1888, not MacMillan.

*Alyssum Schauerianum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

*Synthlipsis lepidota* Rose, Contr. U. S. Nat. Herb. 8: 294. 1905.

Perennial, silvery stellate throughout; stellae small, rays numerous, equal, united for half their lengths or more; caudex woody, sometimes elongating; stems many, branching, procumbent to erect, 1.5–2.5 cm. long; terminal bud developing into a fertile stem; radical leaves narrowly oblanceolate to sublyrate with few teeth, acute, 3–6 cm. long; cauline leaves oblanceolate to nearly linear, entire, repand or with 2–6 sharp teeth, often cuneate at base, 1.5–3.5 cm. long; flowers inconspicuous; petals pale yellow, in age becoming somewhat purplish, 7 mm. long; filaments linear; fruiting inflorescence elongated; pedicels .5–1.5 mm. long, horizontal and sigmoid; pods strongly flattened at right angles to the septum, not keeled, erect, midvein lacking, elliptical or somewhat ovate, sessile, about 1 cm. long, on the mature pod the stellae are not contiguous; styles 1–2 mm. long; stigmas capitate; septum nerved from apex



Fig. 6. *L. Schaueriana*. Habit sketch  $\times \frac{1}{15}$ . Trichomes  $\times 25$ .

more than half way to base, thin, areolae straight or somewhat tortuous; ovules 8–10 in each cell, funiculi attached to septum one-half their lengths or less; seeds neither margined nor winged.

Distribution: Central Mexico.

Specimens examined:

Mexico:

Hidalgo: calcareous soil near Tula, July 13, 1898, *Pringle 6899* (Mo. Bot. Gard. Herb.); near Ixmiquilpan, 1905, *Rose, Painter & Rose 8901* (U. S. Nat. Herb.); near Tula, July 3 and 4, 1905, *Rose, Painter & Rose 8350* (U. S. Nat. Herb.); Hacienda Palmar, near Pachuca, July 21, 1905, *Rose, Painter & Rose 8815* (U. S. Nat. Herb.).

This species is very distinct from any other known member of the genus. On account of the strongly flattened pods it is

associated with *L. lasiocarpa* to which it bears little resemblance in minor details. Petioled stem-leaves as opposed to auriculate ones, elliptical rather than circular pods, and immarginate instead of winged seeds are points of difference between the two species of this section.

No authentic material of Schauer's plant has been seen, but the original description of *Vesicaria argentea* agrees so closely with *Synthlipsis lepidota* Rose that there seems no doubt as to the identity of the type. Watson referred this plant to *Lesquerella* doubtfully, and Gray remarked that when the mature fruit was known it might prove to be a species of *Synthlipsis*. Like *L. lasiocarpa*, however, this plant shows no affinity with *Synthlipsis Greggii* and possesses no characters to keep it out of *Lesquerella*.

4. *L. frigida* (Turcz.) Payson, new comb.

*Vesicaria frigida* Turcz. Bull. Soc. Nat. Moscou 27: 296. 1854.

Cespitose perennial, stellate-pubescent throughout; lower part of the stem unifoliate, the rest naked, sparsely stellate; radical, cauline, and leaves of the sterile shoots tongue-shaped, obtuse, quite entire, grayish silvery; petals unknown; sepals saccate at the base; filaments dilated; placental and valvular nectar glands rather large; terminal raceme many-flowered; pedicels erect, exceeding the pods; pods sessile, valves inflated laterally, sub-impressed in the middle, dorsally 1-nerved; styles half as long as the pods; septum entire, longitudinally nerved; ovules numerous, pendulous, funiculi filiform, almost entirely adnate to the septum; seeds immarginate, compressed parallel to the septum; cotyledons accumbent.

Distribution: collected by *Funck* and *Schlim* in the Sierra Nevada in the province of Merida, Venezuela, at an altitude of 11,000 feet.

This plant is known to me only by the original description from which the above was compiled. The plant is evidently a *Lesquerella* and undoubtedly to be associated with *L. Schaueriana* because of the obcompressed pods.



## SECTION 3. EULESQUERELLA Wats.

§ 3. EULESQUERELLA Wats. in Engler & Prantl. Nat. Pflanzenfam. III. Abt. 2: 1888. 1891.

*Lesquerella* proper. Wats. Proc. Am. Acad. 23: 251. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 117. 1895.

Annuals or perennials, frequently forming conspicuous rosettes; terminal bud developing or inhibited; stems usually unbranched; radical leaves pinnatifid or entire; stem-leaves usually entire; filaments rarely dilated at the base; pods glabrous or stellate, typically inflated, never flattened conspicuously at right angles to the septum, sessile or stipitate; ovules usually few, funiculi attached to the septum for one-half their lengths more or less; seeds rarely winged. Species 5-52.

## KEY TO THE SPECIES

- A. Cauline leaves auriculate.
  - a. Stems conspicuously hirsute at the base; petals 5-7 mm. long..... 5. *L. auriculata*
  - b. Stems scarcely hirsute; petals often 1 cm. long.... 6. *L. grandiflora*
- B. Cauline leaves narrowed at the base.
  - a. Pods glabrous.
    - α. Plants annual.
      - I. Pedicels various but not uniformly recurved, with pods pendent.
        - 1. Pods sessile.
          - \* Ovules 5 or more in each cell; stems rather stout.
            - † Fruiting inflorescence crowded..... 7. *L. densiflora*
            - †† Fruiting inflorescence lax.... 26b. *L. gracilis* var. *sessilis*
          - \*\* Ovules 2 in each cell; stems slender. 24. *L. angustifolia*
        - 2. Pods definitely stipitate.
          - \* Pedicels straight or simply curved, ascending.
            - † Pods globose or oblong, without a shoulder at the base.. 26. *L. gracilis*
            - †† Pods obpyriform, with a shoulder at the base..... 26a. *L. gracilis* var. *repanda*
          - \*\* Pedicels distinctly sigmoid, usually horizontal.
            - † Radical leaves lyrate pinnatifid, with numerous acute segments; stipe over 1 mm. long..... 25. *L. Lindheimeri*
            - †† Radical leaves entire or with few lobes; stipe less than 1 mm. long..... 27. *L. Gordonii*
      - II. Pedicels uniformly recurved; pods more or less pendent.
        - 1. Ovules 4-6 in each cell; Texas species.
          - \* Flowers yellow..... 18. *L. recurvata*
          - \*\* Flowers white..... 19. *L. pallida*

2. Ovules 2 in each cell; coarse New Mexican species..... 20. *L. aurea*
- β. Plants perennial.
- I. Pedicels uniformly recurved; pods more or less pendent.
1. Rays of stellae united only at their bases ..... 14. *L. purpurea*
2. Rays of stellae united for about one-half their lengths ..... 17. *L. pueblensis*
- II. Pedicels straight, simply curved upwards or sigmoid, not uniformly recurved; pods horizontal to erect.
1. Fruiting inflorescence subcorymbose or pods clustered near the apex of the stem.
- \* Styles equalling or exceeding the pods.
- † Radical leaves narrowly oblanceolate ..... 8. *L. Engelmannii*
- †† Radical leaves ovate ..... 9. *L. ovalifolia*
- \*\* Styles much shorter than the pods.
- † Petiole of radical leaves stout; native to Greenland and Labrador ..... 11. *L. arctica*
- †† Petiole slender; native to Newfoundland and Anticosti ..... 11a. *L. arctica* var. *Purshii*
- 2 Fruiting inflorescence elongated.
- \* Pods stipitate, basal leaves pinnatifid ..... 25. *L. Lindheimeri*
- \*\* Pods sessile.
- † Radical leaves gradually narrowed at the base.
0. Ovules 2; stems many, slender ..... 24. *L. angustifolia*
00. Ovules more than 2; stems stouter.
- || Pedicels simply curved, not sigmoid.
- m. Styles usually equalling the pods in length ..... 15. *L. Fendleri*
- n. Styles much shorter than the pods ..... 11. *L. arctica*
- || || Pedicels sigmoid, usually horizontal.
- m. Stellae not scale-like, rays distinct.
- : Leaves distinctly toothed; rays unbranched... 13. *L. argyraea*
- :: Leaves entire; rays branched. 29. *L. pinetorum*
- n. Stellae scale-like, rays united nearly or quite to their apices. 16. *L. Schaffneri*

- †† Radical leaves abruptly narrowed at base; pods oblong 30. *L. pruinosa*
- b. Pods stellate-pubescent.
- α. Pedicels uniformly recurved, not at all sigmoid.
- I. Plants annual; ovules 2..... 30. *L. aurea*
- II. Plants perennial; ovules usually more than 4.
1. Basal leaves linear or oblanceolate.
- \* Stems stout; fruiting racemes not secund..... 21. *L. argentea*
- \*\* Stems slender; fruiting racemes usually secund..... 22. *L. arenosa*
2. Basal leaves oval or suborbicular... 23. *L. macrocarpa*
- β. Pedicels sigmoid, straight or uniformly curved upwards.
- I. Plants annual.
1. Pods globose; stems erect; terminal bud not inhibited.
- \* Pedicels sigmoid; stems usually simple..... 28. *L. Palmeri*
- \*\* Pedicels straight; stems branched. 35. *L. globosa*
2. Pods compressed at the apex; stems decumbent; rosette plants..... 51. *L. Cusickii*
- II. Plants perennial.
1. Radical leaves linear or narrowly oblanceolate.
- \* Fruiting inflorescence raised conspicuously above the leaves.
- † In well-developed plants, pods clustered at apex of stems.
0. Radical leaves thick, usually involute..... 38. *L. intermedia*
00. Radical leaves thinner, flat; stems more slender.
- || Stems with one or more leaves..... 39. *L. arizonica*
- ||| Stems leafless..... 39a. *L. arizonica* var. *nudicaulis*
- †† In well-developed plants, fruiting inflorescence elongated.
0. Pods sessile..... 40. *L. alpina*
00. Pods stipitate..... 42. *L. Garretii*
- \*\* Fruiting inflorescence scarcely raised above the basal leaves.
- † Pubescence spreading..... 41. *L. condensata*
- †† Pubescence closely appressed. 41a. *L. condensata* var. *laevis*
2. Radical leaves oblanceolate, spatulate, oval, or suborbicular.
- \* Pods distinctly stipitate when mature, oblong..... 45. *L. latifolia*
- \*\* Pods sessile.
- † Rays of the stellae unbranched 13. *L. Berlandieri*
- †† Rays of the stellae conspicuously forked.
0. Pods conspicuously elongated, when mature at least twice as long as wide.

- || Pods obtuse or acute,  
but not compressed  
at the apex.
- m. Basal leaf blades  
narrowed gradu-  
ally to the petiole.
- : Pedicels sigmoid;  
stems erect or  
decumbent.
- # Caudex not  
enlarged..... 33. *L. montana*
- # # Caudex large,  
woody..... 33a. *L. montana*  
var. *suffruticosa*
- :: Pedicels rarely  
sigmoid; stems  
prostrate..... 36. *L. mendocina*
- n. Basal leaf blades  
narrowed abrupt-  
ly to the petiole.
- : Stems erect or  
decumbent; pods  
not at all com-  
pressed..... 33. *L. montana*
- :: Stems prostrate;  
pods slightly ob-  
compressed..... 46. *L. Wardii*
- || || Pods compressed at the  
apex; pedicels conspicu-  
ously sigmoid..... 34. *L. curvipes*
- 00. Pods not conspicuously  
elongated.
- || Pods not flattened at  
the apex nor on the  
margins.
- m. Scarcely forming a  
rosette; terminal  
bud developing  
at least a short  
fertile stem.
- : Stems branched;  
ovules 2 in each  
cell..... 35. *L. globosa*
- :: Stems unbranched;  
ovules 5-6 in  
each cell..... 31. *L. lata*
- n. Forming a distinct  
rosette; terminal  
bud inhibited.
- : Radical leaves  
gradually nar-  
rowed to the  
petiole.
- # Stems erect;  
flowers yel-  
low.
- ♂ Tuft of  
radical  
leaves  
raised  
on an  
elongated  
caudex. 10. *L. monte-*

- |    |   |  |
|----|---|--|
| δδ | Rosette sessile.  |  |
| γ  | Ovules 4 or more..  | 32. <i>L. rectipes</i>                         |
| ↗  | Ovules usually 2.....   | 52. <i>L. Douglasii</i>                        |
| ## | Stems prostrate; flowers often red .....                      | 43. <i>L. cinerea</i>                          |
| :: | Radical leaves abruptly narrowed to the petiole.              |  |
| #  | Pods acute at the apex.                                       |  |
| δ  | Radical leaves toothed; often sub-hastate....                 | 48. <i>L. prostrata</i>                        |
| δδ | Radical leaves entire, sub-orbicular..                        | 46. <i>L. Wardii</i>                           |
| ## | Pods obtuse or truncate at the apex.                          |  |
| δ  | Pods sub-globose....  | 44. <i>L. Kingii</i>                           |
| δδ | Pods truncate, ob-compressed.                                 | 47. <i>L. utahensis</i>                        |
|    | Pods flattened at the apex and on the margins.                |  |
| m. | Stems erect or strongly ascending.                            |  |
| :  | Ovules usually 6.....   | 37. <i>L. valida</i>                           |
| :: | Ovules 2-4.   |  |
| #  | Radical leaves acute, stems usually less than 1 dm. long..... | 40a. <i>L. alpina</i><br>var. <i>spatulata</i> |
| ## | Radical leaves obtuse; stems 1-2 dm. long.....                | 50. <i>L. occidentalis</i>                     |
| n. | Stems prostrate or nearly so.                                 |  |
| :  | Caudex densely invested with former leaf-bases, woody...      | 49. <i>L. diversifolia</i>                     |
| :: | Caudex scarcely developed; annual or short-lived perennial.   | 51. <i>L. Cusickii</i>                         |

5. *L. auriculata* (Engelm. & Gray) Wats. Proc. Am. Acad. 23: 250. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 17. 1891; Wats. in Gray, Syn. Fl. N. Am. 1<sup>st</sup>: 116. 1895; Small, Fl. South-eastern U. S. 469. 1903, and ed. 2, 1913.

*Vesicaria auriculata* Engelm. & Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 5: 240. 1847; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 148. 1850; Walp. Ann. 2: 38. 1851.

*Alyssum auriculatum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Annual or biennial, rather sparsely stellate with few-rayed hairs; stems hirsute, particularly at the base, rather stout, 1–2.5 dm. long, few to many from the base, mostly unbranched, decumbent and spreading; terminal bud developing; radical leaves 2–5 cm. long, oblanceolate in outline, lyrate-toothed or subentire, usually obtuse, narrowed at the base but scarcely petioled; cauline leaves shallowly toothed or nearly entire, oblong, 1–3 cm. long, obtuse, sessile and auriculate at the base; flowers yellow; petals 5–7 mm. long; filaments abruptly and broadly dilated at the base; fruiting inflorescence elongated, rather crowded; pedicels ascending-divergent, 1–1.5 cm. long; pods erect, glabrous, sessile, globose, 4–6 mm. in diameter; styles about 2 mm. long; septum thin, areolae somewhat tortuous, nerved from the apex toward the base; ovules 6–8 in each cell, funiculi long and slender, attached to septum for about one-fourth their lengths; seeds narrowly winged.

Distribution: central Oklahoma to southern Texas.

Specimens examined:

Oklahoma: Kingfisher County, April 21, 1896, *L. A. Blankinship* (Rky. Mt. Herb.); Huntsville, Kingfisher County, April 10, 1896, *L. A. Blankinship* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); waste place near Kingfisher, Kingfisher County, April 26, 1913, *Stevens 188* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Texas: *Wright* (U. S. Nat. Herb.); sands, Big Sandy, April 7, 1902, *Reverchon 2967* (Mo. Bot. Gard. Herb.); sands, Terrell, April 5, 1903, *Reverchon 3717* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); sands, southwest of Dawson, April 16, 1903, *Reverchon* (Mo. Bot. Gard. Herb.); on right bank of the Brazos, Feb. 24, 1844, *Lindheimer* (Mo. Bot. Gard. Herb.); near San Felipe on the Brazos, March, 1844, *Lindheimer 217* (Mo. Bot.

Gard. Herb.); probably at San Antonio, 1878, *Ball 1697* (Mo. Bot. Gard. Herb.).

The glabrous pods and the auriculate cauline leaves serve at once to separate this species from all other species of *Lesquerella* except *L. grandiflora*, and from that species it is most easily distinguished by its hirsute stems and smaller flowers. The basal leaves in *L. auriculata*, from the specimens at hand, seem never to become so deeply pinnatifid as in *L. grandiflora*. Ball's specimen labelled "probably at San Antonio" may well be from farther north and east since it has not been confirmed by other collections from this locality.

6. *L. grandiflora* (Hook.) Wats. Proc. Am. Acad. 23: 250. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 17. 1891; Wats. in Gray, Syn. Fl. N. Am. 1': 116. 1895; Small, Fl. Southeastern U. S. 469. 1903, and ed. 2, 469. 1913.

*Vesicaria grandiflora* Hook. Bot. Mag. N. S. 10: t. 3464. 1836; Torr. & Gray, Fl. N. Am. 1: 101. 1838, and suppl. 668. 1840; Don, Sweet's Brit. Fl. Gard. 4: t. 404 1838; Walp. Rep. 1: 141. 1842; Dietr. Syn. Pl. 3: 638. 1843; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 148. 1850; Walp. Ann. 2: 37. 1851.

*V. brevistyla* Torr. & Gray, Fl. N. Am. 1: 102. 1838.

*V. grandiflora* Hook. var. *pinnatifida* Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 146. 1850.

*Alyssum grandiflorum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Rather coarse annual or biennial, loosely stellate, pubescent with few-rayed stellae; stems at the base sparsely villous, erect or decumbent, 2-8 dm. long, simple or sparingly branched; terminal bud developing a fertile stem; radical leaves 3-10 cm. long, oblanceolate or oblong, variously pinnatifid, sometimes merely dentate, often pinnate with equal segments, acute or obtuse, narrowed to a sparingly villous petiole; cauline leaves lanceolate to oblong, conspicuously toothed, 1-3 cm. long, sessile and auriculate at the base; flowers yellow, large; petals often 1 cm. long, broad; filaments gradually dilated at the base; fruiting inflorescence elongated, open; pedicels divergent-ascending, often curved, 1-1.5 cm. long; pods erect, very shortly stipitate, nearly sessile, glabrous, globose or slightly longer than broad, 4-6 mm. in diameter; styles 1-1.5 mm. (rarely 2 mm.).

long, stigmas capitate, conspicuous; septum thin, nerved one-half its length, areolae not tortuous; ovules 4-6 in each cell, funiculi long, slender, attached to septum for about one-fourth their lengths; seeds flat, narrowly winged.

Distribution: south central Texas.

Specimens examined:

Texas: Houston, Harris County, April 10, 1903, *Biltmore Herbarium 14807* (U. S. Nat. Herb.); prairies west of Brazos, April 1, 1839, *Lindheimer* (Mo. Bot. Gard. Herb.); sand hill near Austin, May 15, 1872, *Hall 23* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); sands, Llano, May, 1885, *Reverchon* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); sandy soil, Babyhead, Llano County, May, 1887, *Reverchon* (Mo. Bot. Gard. Herb.); Columbus, April 8, 1907, *Howell 356* (U. S. Nat. Herb.); prairies west of Victoria, Feb., 1845, *Lindheimer* (Mo. Bot. Gard. Herb.); Victoria, April 28, 1905, *Tracy 9193* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); Victoria, April 28, 1905, *Maxon 3815* (U. S. Nat. Herb.); dry open ground, Goliad, Goliad County, March 10, 1916, *Palmer 9136* (Mo. Bot. Gard. Herb.); Herbarium Texano-Mexicanum, *Berlandier 2538* (Mo. Bot. Gard. Herb.).

*L. grandiflora* possesses the largest flowers of any known member of the genus and is quite an attractive plant when in blossom. The specific name *brevistyla* was given by Torrey and Gray to a Texas plant which they believed to be distinct from *L. grandiflora*, judging from the illustration of that species in Sweet's 'British Flower Garden.' Later, with authentic material at hand, they recognized the two as identical. The variety  $\beta$ . *pinnatifida* differs from the species in having more deeply pinnate basal leaves and is probably only an ecological form. *L. grandiflora* is very close to *L. auriculata*. No intermediate specimens have been seen, however, and though the differences are slight the two plants may best retain their specific designa-



Fig 7 *L. grandiflora*. Habit sketch  $\times \frac{1}{3}$  Tr. chomes  $\times 25$ .



tions. The range of the present species seems to be consistently west and south of that of *L. auriculata*.

7. *L. densiflora* (Gray) Wats. Proc. Am. Acad. 23: 251. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 17. 1891; Wats. in Gray, Syn. Fl. N. Am. 1: 120. 1895; Small, Fl. Southeastern U. S. 469. 1903, ed. 2, 469. 1913.

*Vesicaria densiflora* Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 145. 1850; Walp. Ann. 2: 138. 1851; Gray, Smithson. Contr. (Pl. Wright.) 3: 10. 1852.

*Alyssum densiflorum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Annual or biennial; cinerous throughout with rather loose stellae; stellae few-rayed with deep U-shaped fork on one side, rays distinct, long; stems several to many from the base, decumbent to erect, 1-5 dm. long, simple or branched; terminal

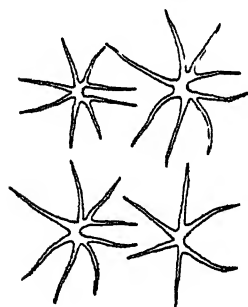


Fig. 8. Trichomes of *L. densiflora*.  $\times 25$ .

bud producing a fertile stem; radical leaves oblanceolate, dentate or lyrate-pinnatifid, 3-7 cm. long; cauline leaves numerous, oblanceolate, usually shallowly toothed, narrowed at the base, 1.5-3 cm. long; petals yellow, narrowed to a slender claw, about 7 mm. long, filaments gradually dilated at the base; fruiting inflorescence short, crowded; pedicels straight, ascending or the lower almost horizontal, usually a little less than 1 cm. long; pods erect, substipitate, globose, glabrous, 3-5 mm. in diameter; styles slender, 4-5 mm. long; stigmas capitate; septum thin, nerved, areolae slightly tortuous; ovules 5-10 in each cell, funiculi long and slender, attached to septum for about three-fourths their lengths; seeds neither margined nor winged.

Distribution: in a narrow area extending north and south across central Texas from Hood to Victoria counties.

Specimens examined:

Texas: 1892, *Nealley* (U. S. Nat. Herb.); sands, Falls Creek, Hood County, April, 1885, *Reverchon* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Comanche Peak near Granbury, May 5, 1900, *Eggert* (Mo. Bot. Gard. Herb.); rocky prairie, near Granbury, May 6, 1900, *Eggert* (Mo. Bot. Gard. Herb.); rocky up-

lands, Somerville County, April, 1882, *Reverchon* (Mo. Bot. Gard. Herb.); Waco, *Pace* (Mo. Bot. Gard. Herb.); Ft. Chadbourn, 1856, *Swift* (U. S. Nat. Herb.) limestone barrens, Brownwood, Brown County, March 31, 1917, *Palmer 11429* (Mo. Bot. Gard. Herb.); dry hills, Austin, May 20, 1872, *Hall 19* (Mo. Bot. Gard. Herb.); sandy open ground, Fredericksburg, Gillespie County, June 5, 1916, *Palmer 10076* (Mo. Bot. Gard. Herb.); Fredericksburg, May 9, 1899, *Bray 285* (U. S. Nat. Herb.); gravelly banks of rivulets near Fredericksburg, May, 1847, *Lindheimer 577* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); sandy soil on the lower Guadalupe, near Victoria, Feb., 1845, *Lindheimer 328* (Mo. Bot. Gard. Herb.); Womack, Victoria County, April 11, 1900, *Eggert* (Mo. Bot. Gard. Herb.); sandy open ground, Victoria, March 13, 1916, *Palmer 9153* (Mo. Bot. Gard. Herb.).

This species is of interest particularly because of the short, crowded inflorescence, a character that is carried a step farther in the subumbellate inflorescence of *L. Engelmannii*. From that species the annual habit also distinguishes *L. densiflora*. *L. argyrea* is similar in many ways to the present species but the former is distinctly perennial, the inflorescence elongated, and the pedicels usually sigmoid. *L. gracilis* has the annual habit of *L. densiflora*, but there again the inflorescence is elongated. In the stellae is to be found a character that serves definitely to separate this species from any other with which it might be confused except *L. Engelmannii*. In these two species the radial symmetry of the star is broken by a deeper U-shaped fork on one side—a character easily understood by comparison of the drawings of the stellae of the species under discussion.

8. *L. Engelmannii* (Gray) Wats. Proc. Am. Acad. 23: 254. 1888; Coulter, Contr. U. S. Nat. Herb 2: 18. 1891; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 120. 1895; Small, Fl. Southeastern U. S. 471. 1903, and ed. 2. 471. 1913; Nelson in Coulter & Nelson, Manual Cent. Rocky Mountains, 219. 1909 (in part); Rydb. Fl. Rocky Mountains, 333. 1917.

*Vesicaria Engelmannii* Gray, Gen. Am. Bor.-Or. Ill. 1: 162. t. 70. 1848; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 144. 1850; Walp. Ann. 2: 39. 1851; Gray, Smithson. Contr. (Pl. Wright.) 3: 110. 1852.

*V. pulchella* Kunth & Bouché, Ann. Sci. Nat. Bot. III. 2: 229. 1849.

*V. Engelmannii* Gray, var. *β. elatior* Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 145. 1850.

*Alyssum Engelmannii* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Perennial, canescent with a coarse, stellate pubescence, stellae rather conspicuously granular roughened, rays simple or forked at the base; caudex branching; stems usually many, erect or slightly decumbent, unbranched, 1.5–4 dm. long, usually lateral, the terminal bud producing a shorter sterile shoot; radical leaves 3–7 cm. long, narrowly lanceolate, acute, the outermost broader, nearly obovate, rarely persisting, all gradually narrowed to a slender petiole; cauline leaves narrowly oblanceolate to nearly linear, narrowed to a slender base, entire; flowers rather showy; petals yellow, spatulate, about 1 cm. long, filaments rather broad, gradually dilated toward the base; fruiting inflorescence typically subumbellate, pedicels nearly straight, from horizontal to erect, 1–1.5 cm. long; pods horizontal to erect, glabrous, globose, 4–7 mm. in diameter, stipe about 1 mm. long; styles slender, exceeding the pods; septum thickish, nerved, areolae not tortuous; ovules 5–6 in each cell, funiculi attached to septum for about three-fourths their lengths.

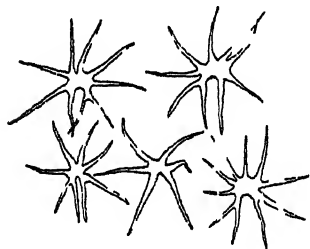


Fig. 9. Trichomes of *L. Engelmannii*.  $\times 25$ .

Distribution: from western Oklahoma south across central Texas.

Specimens examined:

Oklahoma: hillside, Shattuck, Ellis County, May 17, 1914, Clifton 3023 (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Texas: calcareous rocky upland, Dallas, April, 1879, Reverchon (U. S. Nat. Herb.); rocky hills near Dallas, April, 1880, Reverchon (U. S. Nat. Herb.); calcareous soil, Dallas, April 1, 1900, Reverchon (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); calcareous soil near Dallas, April 10, 1900, Reverchon (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); limestone prairies, Five Mile Creek, Dallas County, April 30, 1900, Reverchon (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); dry, stony hills near West

Dallas, May 3, 1900, *Eggert* (Mo. Bot. Gard. Herb.); Hood County, June, 1882, *Reverchon* (U. S. Nat. Herb.); rocky hill, Austin, May 20, 1872, *Hall 21* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Mt. Bonnell, near Austin, April 25, 1914, *Young* (Mo. Bot. Gard. Herb.); pebbly shore of the Guadalupe River, near New Braunfels, May, 1846, *Lindheimer 325* (Mo. Bot. Gard. Herb.); pebbly river banks, New Braunfels, May, 1848, *Lindheimer 576* (Mo. Bot. Gard. Herb.); New Braunfels, May, 1850, *Lindheimer 421* (Mo. Bot. Gard. Herb.); New Braunfels, May, 1850, *Lindheimer 667* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); New Braunfels, April, 1851, *Lindheimer 526* (Mo. Bot. Gard. Herb.); New Braunfels, April, 1851, *Lindheimer 666* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

This species and its northern relative, *L. ovalifolia*, are peculiar among the glabrous podded species in having typically a sub-umbellate inflorescence. Occasionally forms of *L. Engelmannii* occur, however, that have the usual elongated raceme of the genus. To this variation Gray gave the name var.  $\beta$ . *elatior*. The type of *V. pulchella* probably also exhibits this character. Since, however, plants with an elongated flower cluster do not seem to be limited to any range, this variation has not been deemed worthy of taxonomic recognition.

*L. Engelmannii* is probably the most conspicuous member of the genus when in blossom, due in part to the large size of the flowers and in part to the manner in which they are clustered at the apices of the erect stems. The distribution of this species is evidently limited to calciferous soils.

9. *L. ovalifolia* Rydb. in Britton & Brown, Ill. Fl. 2: 137. 1897, and ed. 2, 2: 156. 1913; Rydb. Fl. Colo. 155. 1906; Petersen, Fl. Nebraska, 62. 1912; Wooton & Standley, Contr. U. S. Nat. Herb. 19: 276. 1915, in part; Rydb. Fl. Rocky Mountains, 333. 1917.

*L. ovata* Greene, Pittonia 4: 308. 1901.

*L. Engelmannii* Nelson in Coulter & Nelson, Manual Cent. Rocky Mountains, 219. 1909.

Perennial, densely silvery stellate, stellae many-rayed, rays simple or branching, crowded, granular roughened; caudex frequently much branched; stems erect or decumbent, .5-2 dm.

long, unbranched, lateral, terminal bud remaining undeveloped or rarely elongating to form a short sterile shoot; radical leaves

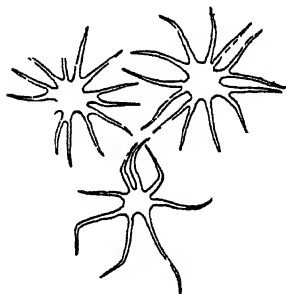


Fig. 10. Trichomes of *L. ovalifolia*.  $\times 25$ .

broadly oblanceolate to nearly orbicular, entire, blade .5-2 cm. long, abruptly narrowed to a slender petiole 5-25 mm. long; cauline leaves linear-oblanceolate, rather few, entire; petals yellow, frequently over 1 cm. long, spatulate, filaments narrow, broader toward the base; fruiting inflorescence typically contracted and subumbellate, pedicels

ascending or erect, .5-1.5 cm. long, pods ascending to erect, substipitate, glabrous, globose or slightly oblong, 4-5 mm. in diameter, style slender, equalling or exceeding the pod; septum nerved, areolae not tortuous; ovules 5-8 in each cell, funiculi attached for more than half their lengths.

Distribution: western Oklahoma and Kansas, southwestern Nebraska, northern Texas, northeastern New Mexico, and southeastern Colorado.

Specimens examined:

Nebraska: hills of upper Lawrence Fork, Kimball County, Aug. 11, 1891, *Rydberg 22* (U. S. Nat. Herb.).

Kansas: gypsum hills, Rooks County, June 20, 1897, *Hitchcock 1077* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); Coolidge, July, 1892, *Hitchcock* (Mo. Bot. Gard. Herb.).

Oklahoma: grassy mountain side, near Crusher Spur, Murray County, April 12, 1913, *Stevens 36* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); Arbuckle Mountains, Davis, April 1, 1916, *Emig 498* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); dry gravelly banks, common, near Tishomoningo, Johnston County, April 15, 1916, *Houghton 3573* (Mo. Bot. Gard. Herb.); rough hillside, near Knowles, Beaver County, May 6, 1913, *Stevens 348* (Mo. Bot. Gard. Herb.).

Texas: rocky bluffs, Amarillo Creek, May 28, 29, 1902, *Reverchon* (Mo. Bot. Gard. Herb.); rocky bluffs, Galaduro, May 30, 1902, *Reverchon* (Mo. Bot. Gard. Herb.); rocky bluffs on Red River, Randall County, June 9, 1901, *Eggert* (Mo. Bot. Gard. Herb.); rocky bluff near Canyon City, Randall County, June 10,

1901, *Eggert* (Mo. Bot. Gard. Herb.); dry open ground, Canyon, Randall County, July 12, 1917, *Palmer 12526* (Mo. Bot. Gard. Herb.); plains and prairies, Post City, March 17, 1909, *Ruth 5* (U. S. Nat. Herb.); Glover's Pasture, Grady, Fisher County, April 14, 1901, *Shepherd* (U. S. Nat. Herb.).

Colorado: Rule Creek, Bent County, May 22, 1913, *Osterhout 4878* (Geo. Osterhout Herb.); bluffs of Arkansas at Pueblo, May, 1873, *Greene* (Mo. Bot. Gard. Herb.); mesas near Pueblo, May 14, 1900, *Rydberg & Vreeland 6142* (Rky. Mt. Herb.); Pueblo, May 23, 1914, *Bethel* (Rky. Mt. Herb.).

New Mexico: stony hills, Nara Visa, April 21, 1911, *Fisher 104* (U. S. Nat. Herb.).

*L. ovalifolia*, because of its contracted inflorescence, is likely to be confused with no other species except *L. Engelmannii* and to this species it is most closely related. The broader basal leaves, whose blades are abruptly narrowed to the petiole, and the denser, more silvery pubescence serve to give *ovalifolia* a different appearance. It is also a lower, more compact plant and shows the rosette habit quite definitely established. In *L. Engelmannii* the terminal bud normally develops a sterile shoot several centimeters in length. The ranges of the two species are consistently separated. *L. ovalifolia* is undoubtedly a distinct calciphyte.

**10. *L. montevidensis* (Eichl.) Wats.** Proc. Am. Acad. 23: 251. 1888.

*Vesicaria montevidensis* Eichl. in Mart. Fl. Bras. 13: 302. *t.* 67, fig. 2. 1865; Gilg & Muschler in Engl. Bot. Jahrb. 42: 466. 1909.

Perennial, silvery stellate-pubescent throughout, stellae many-rayed, rays forked at the base, distinct or irregularly coherent; caudex woody, branched, somewhat elongated; stems erect or decumbent, 3-6 dm. long; terminal bud remaining undeveloped or producing only a short sterile shoot; radical leaves oblanceolate, remotely dentate or subentire, 2.5-3.5 cm. broad, petiole short; cauline leaves narrowly oblanceolate, remotely dentate or subentire, acute; petals yellow, obovate; filaments slightly dilated at the base; fruiting inflorescence rather short; pedicels straight or simply curved, ascending; pods erect, sessile, ellip-

soid, 6-7 mm. long, 4-5 mm. broad, sparsely stellate-pubescent; styles 3-4 mm. long; septum nerved; ovules 3-4 in each cell; seeds not winged.

Distribution: Uruguay.

No specimens of this plant have been seen, but the figure in the 'Flora Brasiliensis' is so detailed, little doubt remains that this species is properly placed under *Lesquerella*. In appearance it is not unlike *L. Engelmannii* but its affinity to this species is of course merely conjectural.

11. *L. arctica* (Wormsk.) Wats. Proc. Am. Acad. 23: 254. 1888; Wats. in Gray, Syn. Fl. N. Am. 1: 120. 1895; Britton & Brown, Ill. Fl. 2: 138. 1897, and ed. 2, 2: 156. 1913; Simmons, Rept. Second Norwegian Arctic Exp. in the Fram, No. 2, 95. 1906.

*Alyssum arcticum* Wormsk. ex Horne. Fl. Dan. 9: t. 1520. 1814.

*A. ? arcticum* DC. Syst. 2: 324. 1821.

*Vesicaria arctica* Richards in Frankl. Narr. First Jour. App. 743. 1823; Hook. Fl. Bor. Am. 1: 48. 1829; Torr. & Gray, Fl. N. Am. 1: 100. 1838; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 149. 1850; Durand, Jour. Acad. Nat. Sci. Phila. 3: 186. 1856; Busch, Zweite Deutsche Nordpolarfahrt 2, Abt. I: 30. 1874; Macoun, Cat. Canadian Pl. 1: 54. 1883; Meehan, Proc. Acad. Nat. Sci. Phila. 1893: 208. 1893.

Perennial, silvery stellate throughout with a dense scurfy pubescence; stellae small, many-rayed, rays confluent at the base; stems lateral, decumbent or nearly erect, 1-2 dm. long, unbranched; terminal bud remaining undeveloped; radical leaves densely rosulate on the thick caudex, 1-5 cm. long, thick, from spatulate to narrowly oblanceolate, entire, obtuse or acute, petiole broad; cauline leaves few, linear oblanceolate, entire, 8-15 mm. long, tapering to a narrow base; flowers rather few; petals yellow, 5-6 mm. long, scarcely narrowed to a claw; filaments rather stout, linear; fruiting inflorescence open, showing a tendency to remain corymbose; pedicels stout, erect or ascending, about 1.5 cm. long; pods erect, glabrous, sessile, globose or slightly elongated, 5-6 mm. in diameter; styles 1-2 mm. long, stigmas enlarged; septum thin, often perforate, nerved, areolae

slightly tortuous; ovules 6-8 in each cell, flat, not winged, funiculi rather short, attached to septum for about one-half their lengths.

Distribution: Greenland, Ellesmereland, and the arctic coast of America at least as far west as the Mackenzie River; extending southward on the coast of Labrador.

Specimens examined:

Greenland: *Drejer* (Mo. Bot. Gard. Herb.); Nettiik, Aug. 4, 1861, *Hayes 10* (U. S. Nat. Herb.); Itiblu Whale Sound, 1891, *Burk 7* (U. S. Nat. Herb.); Borden Bay, Aug. 25, 1901, *Stein 174* (U. S. Nat. Herb.); Borden Bay, Aug. 26, 1901, *Stein 176* (U. S. Nat. Herb.); northeast coast about 76° 45' N. Lat., June 6, 1908, *Andr. Lundarr* (U. S. Nat. Herb.); Kangerdluarsuk kingua 74° 18', July 28, 1887, *Ryder* (Mo. Bot. Gard. Herb.); Nugsuak Patut, July, 1909, *Porsild* (Mo. Bot. Gard. Herb.).

11a. Var. *Purshii* Wats. Proc. Am. Acad. 23: 254. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 120. 1895; Britton & Brown, Ill. Fl. 2: 138. 1897, and ed. 2, 2: 156. 1913; Fernald, Rhodora 13: 223. 1911.

A more slender plant than the species and with narrower leaves. Watson described the variety as having an entire septum in contradistinction to the species which was thought to have a perforate septum. This character is apparently of no value, since specimens of typical *arctica* are at hand that show no perforation. Frequently a few scattering stellae may be found on the otherwise glabrous pods, a character occurring more rarely in the species. From available material the variety seems to have but 5-6 ovules in each cell, while the species has 6-8. This, of course, is of slight value for identification but is of interest in its phylogenetic significance. This plant (var. *Purshii*) is, according to Fernald, a typical calciphile. Few flowering plants penetrate nearer the pole than *L. arctica*.

Distribution: Anticosti Island in the Gulf of St. Lawrence and western Newfoundland.

Specimens examined:

Newfoundland: dry limestone barren, Table Mountain, Port au Port Bay, July 16 and 17, 1914, *Fernald & St. John 216* (Mo. Bot. Gard. Herb., Deam Herb., and Rky. Mt. Herb.); dry lime-



stone barrens, Table Mountain, region of Port au Port Bay, Aug. 16, 1910, *Fernald & Wiegand 3465* (Rky. Mt. Herb. and Mo. Bot. Gard. Herb.).

12. *L. argyraea* (Gray) Wats. Proc. Am. Acad. 23: 254. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 18. 1891; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 120. 1895; Small, Fl. Southeastern U. S. 471. 1903, and ed. 2, 471. 1913.

*Vesicaria argyraea* Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 146. 1850; Walp. Ann. 2: 39. 1851; Wats. Proc. Am. Acad. 17: 319. 1852.

*V. recurvata* Gray, Smithson. Contr. (Pl. Wright.) 5: 13. 1853.

*Alyssum argyreum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Perennial, canescent with coarse stellae, rays few to many, distinct or slightly united at the base, unbranched; stems spreading, procumbent, 1-5 dm. long, usually unbranched; terminal bud remaining undeveloped; radical leaves entire, repand or lyrate, oblanceolate, 2-6 cm. long, narrowed to a slender petiole at the base; cauline leaves very numerous, from very narrowly oblanceolate to ovate, usually toothed, narrowed at the base, 1-4 cm. long; petals yellow, broadly spatulate, 6-9 mm. long; filaments linear; fruiting inflorescence elongated; pedicels horizontally spreading, usually more or less sigmoid by the upwardly curved apex, 1-2 cm. long; pods erect, sessile, glabrous, globose, 3-5 mm. in diameter; styles 2-4 mm. long, stigmas capitate; septum thin, nerved, areolae slightly or not at all tortuous; ovules 8-16 in each cell, funiculi long and slender, attached to septum for more than one-half their lengths; seeds small, not winged.

Distribution: southwestern Texas and northeastern Mexico.

Specimens examined:

Texas: Llano, May 12-16, 1899, *Bray 304* (U. S. Nat. Herb.); Llano County, May, 1884, *Reverchon* (Mo. Bot. Gard. Herb.); Llano, May, 1888, *Reverchon 1489* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Jim Creek, Gillespie County, *Jermy 244* (Mo. Bot. Gard. Herb.); New Braunfels, April, 1850, *Lindheimer 670* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); New Braunfels, April, 1850, *Lindheimer 367* (Mo. Bot. Gard. Herb.); Cuero,

March 22, 1907, *Howell 332* (U. S. Nat. Herb.); prairie near Victoria, Feb., 1845, *Lindheimer 303* (Mo. Bot. Gard. Herb.); sandy banks of Green Lake near the mouth of Guadalupe, Feb., 1845, *Lindheimer 329* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); shell marl banks near bay, Corpus Christi, March 8, 1917, *Palmer 11219* (Mo. Bot. Gard. Herb.); rocks near Goliad, April 9, 1900, *Eggert* (Mo. Bot. Gard. Herb.); sandy open ground, Refugio, March 9, 1916, *Palmer 9121* (Mo. Bot. Gard. Herb.); Brackett, March 21, 1900, *Trelease 21* (Mo. Bot. Gard. Herb.); Ft. Clark, Kinney County, March 22, 1893, *Mearns 1336* (U. S. Nat. Herb.); Ft. Clark, Kinney County, Feb. 27, 1893, *Mearns 1246* (U. S. Nat. Herb.); Eagle Pass, April, 1883, *Havard* (U. S. Nat. Herb.); sands, Laredo, March 29, 1903, *Reverchon 3718* (Mo. Bot. Gard. Herb.); sandy banks of Rio Grande, Webb County, April 6, 1901, *Eggert* (Mo. Bot. Gard. Herb.); stony prairies on the Pinto Creek, western Texas, May, 1851, *Wright 849* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); valley of the Rio Grande, *Parry, Bigelow, Wright & Schott 43* (U. S. Nat. Herb.); western Texas, Oct., 1849, *Wright 15* (U. S. Nat. Herb.).

Mexico:

Nuevo Leon: Monterey and Pico Chico, March 18, 1900, *Canby 21* (U. S. Nat. Herb.); Monterey, Feb. 20, 1900, *Trelease 22* (Mo. Bot. Gard. Herb.).

Coahuila: valley of the Rio Grande near Piedras Negras, April 27, 1900, *Pringle 9182* (U. S. Nat. Herb.); Sabinas, May 21, 1902, *Nelson 6771* (U. S. Nat. Herb.); Saltillo, March 3, 1847, *Gregg 292* (Mo. Bot. Gard. Herb.); Sierra Madre, 40 miles north of Saltillo, July 25-31, 1880, *Palmer 30* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Saltillo, 1898, *Palmer 182* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Chojo Grande, 27 miles southeast of Saltillo, Aug. 29, 1904, *Palmer 372* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); battlefield near Buenavista, March 19, 1847, *Gregg 315* (Mo. Bot. Gard. Herb.); Buenavista, Feb. 14, 1847, *Gregg 90* (Mo. Bot. Gard. Herb.); Parras, March, 1905, *Purpus 1024* (Baker Herb. at Pomona College, and Mo. Bot. Gard. Herb.).

San Luis Potosi: Rio Verde, June 2-8, 1904, *Palmer 464* (U. S. Nat. Herb.); in the region of San Luis Potosi, 1878, *Parry & Palmer 25* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.);

Minas de San Rafael, Nov., 1910, *Purpus* 4920 (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

The outstanding characteristics of this species are the coarse pubescence with the unbranched rays, the perennial habit, the numerous cauline leaves, the doubly curved pedicels, the glabrous, sessile pods, and the numerous ovules. Among the annual species it is sometimes confused with *L. gracilis* or *L. Gordonii*. From both of these the sessile pods serve to separate it. The absence of a gynophore also distinguishes it from *L. Lindheimeri* of the biennial or perennial species.

13. *L. Berlandieri* (Gray) Wats. Proc. Am. Acad. 23: 252. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 118. 1895.

*Vesicaria Berlandieri* Gray, in herb.; Wats. Bibliog. Ind. N. Am. Bot. 1: 75. 1878 (name only).

*Alyssum Berlandieri* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Perennial, silvery canescent throughout, stellae closely contiguous, rays few to many, simple, distinct from near the base; stems slender, procumbent or ascending, 1-3 dm. long, simple or branched; terminal bud remaining undeveloped; radical leaves 2-8 cm. long, entire, undulate or lyrate with very large terminal lobes, obtuse, narrowed at the base to a slender petiole; cauline leaves 1.5-2.5 cm. long, sinuately dentate to nearly entire,



Fig. 11. *L. Berlandieri*. Habit sketch  $\times \frac{1}{2}$ . Trichomes  $\times 25$ .

oblanceolate or broader, narrowed to a slender petiole; petals yellow, sometimes apparently turning reddish on fading, spatulate; filaments linear; fruiting inflorescence elongated; pedicels sigmoid, horizontal or somewhat recurved, 8-15 mm. long; pods erect,

stellate-pubescent, sessile, subglobose or oblong, about 4 mm. in diameter; styles slender, 3-4 mm. long; septum nerved, areolae not tortuous; ovules 4-7 in each cell, funiculi attached for about half their lengths; seeds neither winged nor margined.

Distribution: State of Tamaulipas, Mexico.

Specimens examined:

Mexico:

Tamaulipas: near Matamoras, April, 1831, *Berlandier* 884, 2314 (Gray Herb. TYPE); San Fernando, Oct., 1830, *Berlandier* 819, 2239 (Gray Herb.); Soto la Marina, March 2, 1902, *Nelson* 6631 (U. S. Nat. Herb.).

This species finds its nearest relative in *L. argyrea* and from it is at once separated by the stellate pods and the reduced number of ovules. *L. Berlandieri* is of especial interest because it is the only species of this group that has developed a stellate-pubescent pod. In aspect it is not unlike certain species of the Rocky Mountain region but is easily distinguished from any of them by the simple rays of the stellae.

14. *L. purpurea* (Gray) Wats. Proc. Am. Acad. 23: 253. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 17. 1891; Wats. in Gray, Syn. Fl. N. Am. 1': 119. 1895; Wootton & Standley, Contr. U. S. Nat. Herb. 19: 275. 1915; Armstrong, Field Book of Western Wild Flowers, 184. 1915.

*Vesicaria purpurea* Gray, Smithsonian. Contr. (Pl. Wright.) 5: 14. 1853; Walp. Ann. 4: 196. 1857; Torr. Bot. Mex. Bc ind. Survey, 33. 1859.

*V. purpurea* Gray var. *albiflora* Torr. Bot. Ives' Rept. 6. 1860.

*Alyssum purpureum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Perennial, silvery stellate throughout, rays usually not forked, numerous, slightly coherent at base; stems decumbent or erect, 1-4 dm. long, simple or sparingly branched; terminal bud

remaining undeveloped or producing only a short sterile shoot; radical leaves oblanceolate to oval, entire, repand or lyrate



Fig. 12. *L. purpurea*. Habit sketch  $\times \frac{1}{8}$ . Trichomes  $\times 25$ .

pinnatifid, obtuse, 2-12 cm. long, narrowed at base into a slender petiole; cauline leaves rather remote, oblanceolate, entire, acute or obtuse, 7-25 mm. long; petals white, pink or purplish, 7-9 mm. long, blade narrowed to a distinct claw; filaments slightly broader at the base; fruiting inflorescence elongated; pedicels slender, horizontal to recurved, 6-15 mm. long; pods horizontal to pendent, sessile, glabrous, globose, 4-6 mm. in diameter; styles about 2 mm. long; septum strongly nerved, areolae inclined to be tortuous; ovules 2-5 in each cell, funiculi short, attached to septum for about one-half their lengths; seeds flat, not winged.

Distribution: southwestern Texas, southern New Mexico, southeastern Arizona, northern Sonora, Chihuahua and Coahuila.

Specimens examined:

Texas: Chenates region, 1889, *Nealley 477* (U. S. Nat. Herb.); mouth of Pecos River, Oct., 1883, *Havard* (U. S. Nat. Herb.); Langtry, Val Verde County, Oct., 1892, *Nealley 121a* (U. S. Nat. Herb.); Roma, Starr County, 1899, *Nealley 268* (U. S. Nat. Herb.); stony hills near El Paso, March and April, 1852, *Wright 1320* (Mo. Bot. Gard. Herb.); El Paso, 1881, *Vasey* (U. S. Nat. Herb.); El Paso, April 17, 1884, *Jones 3722* (U. S. Nat. Herb.); dry hills, vicinity of El Paso, 1911, *Stearns 137* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

New Mexico: Organ Mountains, May 15, 1892, *Wooton* (U. S. Nat. Herb.); Filmore Canyon, Organ Mountains, Aug. 4, 1895, *Wooton* (U. S. Nat. Herb.); Organ Mountains, Sept. 4, 1897, *Wooton* (U. S. Nat. Herb.); Organ Mountains, April 15, 1899, *Wooton* (U. S. Nat. Herb. and Deam Herb.); Organ Mountains, March 18, 1900, *Wooton* (U. S. Nat. Herb.); Organ Mountains, April 4, 1903, *Wooton* (U. S. Nat. Herb.); Bishop's Cap, Organ Mountains, March 30, 1905, *Wooton* (U. S. Nat. Herb.); Van Pattens, Organ Mountains, June 10, 1906, *Standley* (U. S. Nat. Herb.); Organ Mountains, June 9, 1906, *Standley* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Florida Mountains, March 7, *Herrick 304* (U. S. Nat. Herb.); Big Hatchet Mountain, May 18, 1892, *Mearns 4* (U. S. Nat. Herb.).

Arizona: common in the Huachuca Mountains near Igo's ranch, April 11, 1908, *Tidestrom 827* (U. S. Nat. Herb.); brushy

slopes, Bisbee, Mule Mountains, April, 1909, *Goodding 61* (Rky. Mt. Herb.); gravelly hills, San Francisco Mountains, March 21, 1881, *Rusby 15* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); Lowell, May, 1884, *Parish* (U. S. Nat. Herb.); Sanoita Valley, April, 1880, *Lemmon* (U. S. Nat. Herb.); Sabina Canyon, March 20, 1897, *Zuck* (U. S. Nat. Herb.); Santa Catalina Mountains, May 14, 1883, *Pringle* (U. S. Nat. Herb.); Santa Rita Forest Reserve, March 31–April 23, 1903, *Griffiths 4146* (U. S. Nat. Herb.); Willow Spring Mountains, March 13–April 23, 1903, *Griffiths 3646* (U. S. Nat. Herb.); shaded places, Sierra Tucson, April 29, 1884, *Pringle* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Agua Verde Creek, April 23, 1914, *Harris C. 1485* (U. S. Nat. Herb.); canyons near Camp Grant, April 2, 1867, *Palmer* (Mo. Bot. Gard. Herb.).

Mexico:

Chihuahua: shaded ledges, hills near Chihuahua, Oct. 24, 1886, *Pringle 949* (U. S. Nat. Herb.); vicinity of Chihuahua, April 8–27, 1908, *Palmer* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); Santa Eulalia Hills, July 30, 1885, *Wilkinson* (U. S. Nat. Herb.); Santa Eulalia Mountains, April 4, 1908, *Rose 11692* (U. S. Nat. Herb.); vicinity of Cusihuiriachic, April 2 and 3, 1908, *Rose 11652* (U. S. Nat. Herb.).

*L. purpurea* is one of the most distinctive species of the genus and is likely to be confused with no other species. Its distinguishing characteristics are the perennial root with the rosette of broad, silvery leaves, the white or purplish flowers, and the glabrous pods on pedicels that are normally recurved in age. It apparently flowers frequently in the late summer or fall.

15. *L. Fendleri* (Gray) Wats. Proc. Am. Acad. 23: 254. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 18. 1891; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 120. 1895; Nelson in Coulter & Nelson, Manual Cent. Rocky Mountains, 219. 1909; Clements & Clements, Rocky Mountain Flowers, 25. 1914; Wootton & Standley, Contr. U. S. Nat. Herb. 19: 276. 1915.

*Vesicaria Fendleri* Gray, Mem. Am. Acad. N. S. (Pl. Fendl.) 4: 9. 1849; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 149. 1850; Walp. Ann. 2: 39. 1851; Torr. & Gray, Pac. Rail. Rept. 2: 160. 1855; Torr. Pac. Rail. Rept. 5: 66. 1856; Coulter,

Manual Rocky Mountain Region, 25. 1885.

*V. stenophylla* Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 149. 1850; Walp. Ann. 2: 39. 1851; Gray, Smithson. Contr. (Pl. Wright.) 3: 10. 1852; Walp. Ann. 4: 196. 1857; Porter & Coulter, Syn. Fl. Colo. 6. 1874.

*V. stenophylla* Gray, vars.  $\beta$ . *procera*,  $\gamma$ . *siliculis ovatis*,  $\delta$ . *humilis* and  $\epsilon$ . *diffusa* Gray, Smithson. Contr. (Pl. Wright.) 5: 13. 1853.

*Alyssum Fendleri* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

*Lesquerella foliacea* Greene, Pittonia 5: 134. 1903.

*L. stenophylla* Rydb. Bull. Torr. Bot. Club 33: 142. 1906; Rydb. Fl. Colo. 155. 1906; Rydb. Fl. Rocky Mountains, 333. 1917.

*L. praecox* Wooton & Standley, Contr. U. S. Nat. Herb. 16: 126. 1913; Wooton & Standley, Contr. U. S. Nat. Herb. 19: 276. 1915.

Perennial, caudex usually branched; plant silvery stellate throughout with closely overlapping stellae, rays numerous, simple, confluent from one-third to two-thirds their lengths; stems simple or branching, tufted, mostly erect, .5-3 dm. long, leafy; terminal bud developing a fertile stem; radical and cauline leaves similar, linear to linear-oblongate, entire or variously toothed, 1-4 cm. long, narrowed to a slender base; petals yellow, often nearly 1 cm. long, broadly spatulate; filaments filiform; fruiting inflorescence elongated or short and crowded, sometimes scarcely exceeding the leaves; pedicels erect or strongly ascending, .5-2 cm. long; pods erect, sessile, glabrous, globose or elongated, 3-7 mm. in diameter; styles 2-6 mm. long, slender; stigmas capitate; septum thin, nerved, areolae not tortuous; ovules 8-16 in each cell, funiculi long and slender, attached to the septum for one-half their lengths or more; seeds not margined.

Distribution: southwestern Kansas, western Texas, southeastern Colorado, New Mexico, southeastern Utah, western Arizona, and north central Mexico.

Specimens examined:

Kansas: without definite locality, 1876, *Popenoe* (U. S. Nat. Herb.).

Texas: Spafford Junction, March 22, 1900, *Canby* 18 (U. S. Nat. Herb.); near Cormidos, Nov., 1881, *Havard* (U. S. Nat.

Herb.); Davis Mountains, April 29, 1902, *Tracy & Earle 338* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); dry, rocky, open ground, Boerne, Kendall County, April 6, 1917, *Palmer 11479* (Mo. Bot. Gard. Herb.); Canyon City, June 10, 1901, *Eggert* (Mo. Bot. Gard. Herb.); sandy bluffs, upper Concho, April, 1882, *Reverchon* (U. S. Nat. Herb.); middle fork of Concho, April, 1882, *Reverchon* (Mo. Bot. Gard. Herb.); rocky prairies near Big Spring, Howard County, June 11, 1900, *Eggert* (Mo. Bot. Gard. Herb.); Big Spring, May 12, 1902, *Tracy 8044* (Mo. Bot. Gard. Herb.); rocky places on the south Llano, June, 1884, *Reverchon* (Mo. Bot. Gard. Herb.); dry bluffs, upper Llano, June, 1885, *Reverchon* (U. S. Nat. Herb.); prairies at the head of the Limpio, June, 1851, *Wright 852* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); bluffs of Pecos River, Sept., 1881, *Havard* (U. S. Nat. Herb.); plains west of Pecos, April 20, 1902, *Tracy & Earle 143* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); vicinity of Pecos City, Oct. 14, 1913, *Rose & Fitch 17908* (U. S. Nat. Herb.); Barstow, April 15, 1902, *Tracy & Earle 31* (U. S. Nat. Herb.); near Burgess water hole, W. Texas, July, 1883, *Havard 72* (U. S. Nat. Herb.); plains west of Guadalupe Mountains, Nov., 1881, *Havard* (U. S. Nat. Herb.); rocky prairies on Turkey Creek, May, 1851, *Wright 850* (Mo. Bot. Gard. Herb.); western Texas to El Paso, Oct., 1849, *Wright 16* (U. S. Nat. Herb.); stony hills near El Paso, March, 1852, *Wright 1319* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); El Paso, 1881, *Vasey* (U. S. Nat. Herb.); rocky hills near Van Horn, El Paso County, July 9, 1900, *Eggert* (Mo. Bot. Gard. Herb.); sandy ground near Sierra Blanca, May 15, 1901, *Eggert* (Mo. Bot. Gard. Herb.); "Camp Charlotte," W. Texas, 1889, *Nealley 700, 701* (U. S. Nat. Herb.); near Rio Grande, June, 1904, *Jermy* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Colorado: Rule Creek, Bent County, June 10, 1910, *Osterhout 4412* (Geo. Osterhout Herb.); La Junta, June 19, 1909, *Osterhout 3965* (Geo. Osterhout Herb.); mesas near Pueblo, May 14, 1900, *Rydberg & Vreeland 6143* (Rky. Mt. Herb.); Swallow's, between Pueblo and Canyon City, June 1, 1901, *Baker 2* (Mo. Bot. Gard. Herb., Rky. Mt. Herb., Geo. Osterhout Herb., Baker Herb. at Pomona College, and U. S. Nat. Herb.); Canyon City, 1872, *Brandeggee 345* (Mo. Bot. Gard.



Herb.); Brantly Canyon, Las Animas County, June 10, 1900, *Osterhout 2050* (Rky. Mt. Herb., Geo. Osterhout Herb., and Baker Herb. at Pomona College).

New Mexico: without definite locality, 1851, *Wright 851* (U. S. Nat. Herb.); McArty's Ranch, Aug. 6, 1880, *Rusby 16* (Mo. Bot. Gard. Herb.); vicinity of Farmington, San Juan County, July 17, 1911, *Standley 7091* (U. S. Nat. Herb.); Nara Visa, April 19, 1911, *Fisher 105* (U. S. Nat. Herb.); smaller hills around Santa Fe, May 2, 1847, *Fendler 40* (Mo. Bot. Gard. Herb.); dry, gravelly hills, Santa Fe, May 25, 1847, *Fendler 39* (Mo. Bot. Gard. Herb.); on hills at Santa Fe, May 13, 1897, *Heller 3516* (Baker Herb. at Pomona College); Acoma, May, 1882, *Bandelier* (Mo. Bot. Gard. Herb.); Kelly, May 13, 1895, *Herrick 537* (U. S. Nat. Herb.); Gallinas Mountains, Aug. 27, 1904, *Wooton* (U. S. Nat. Herb.); Roswell, Chaves County, April 18, 1898, *Skehan 3* (Mo. Bot. Gard. Herb., Rky. Mt. Herb., Geo. Osterhout Herb., and U. S. Nat. Herb.); Arroyo Ranch near Roswell, May 4-9, 1903, *Griffiths 4250* (Mo. Bot. Gard. Herb.); Fort Smith to the Rio Grande, 1853-4, *Bigelow* (U. S. Nat. Herb.); road to Apache Teju, Aug. 3, 1895, *Mulford 614* (Mo. Bot. Gard. Herb.); Queen, Aug. 1, 1909, *Wooton* (U. S. Nat. Herb.); in the valley of the Rio Grande, below Dona Ana, *Parry, Bigelow, Wright & Schott 42* (U. S. Nat. Herb.); Tortugas Mountain, Dona Ana County, April 22, 1894, *Wooton* (U. S. Nat. Herb.); Organ Mountains, Dona Ana County, July 15, 1897, *Wooton 155* (Mo. Bot. Gard. Herb., Rky. Mt. Herb., and U. S. Nat. Herb.); Filmore Canyon, Organ Mountains, April 29, 1899, *Wooton* (U. S. Nat. Herb.); Filmore Canyon, Organ Mountains, April 15, 1899, *Wooton* (U. S. Nat. Herb.); Organ Mountains, April 24, 1900, *Wooton* (Rky. Mt. Herb.); mesa west of the Organ Mountains, March 17, 1900, *Wooton* (U. S. Nat. Herb.); mesa west of Organ Mountains, March 2, 1902, *Wooton* (Deam Herb.); Bishop's Cap, Organ Mountains, March 30, 1905, *Wooton* (U. S. Nat. Herb.); Tortugas Mountain, Mesilla Park, May 6, 1906, *Standley* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Mesilla Valley, Dona Ana County, April 8, 1907, *Wooton & Standley* (Mo. Bot. Gard. Herb.); near the Cueva, Organ Mountains, April 25, 1907, *Wooton & Standley* (U. S. Nat. Herb.); Carrizallito Mountains, April 20, 1892, *Mearns 3* (U. S. Nat. Herb.).

Utah: Barton's Range, San Juan County, July 16, 1895, *Eastwood 8* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); canyon above Tropic, May 28, 1894, *Jones 5302a* (U. S. Nat. Herb.).

Arizona: Andrade, March 13–April 23, 1903, *Griffiths 4074* (Mo. Bot. Gard. Herb.); Holbrook, Oct. 9, 1897, *Zuck* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Holbrook, July 30, 1896, *Zuck* (U. S. Nat. Herb.); Holbrook, 1901, *Hough 59* (U. S. Nat. Herb.); 5 miles northeast of Holbrook, June 20, 1901, *Ward* (U. S. Nat. Herb.); near Springerville, June 25, 1892, *Wooton* (U. S. Nat. Herb.); rocky slopes near Douglas, May, 1907, *Goodding 2228* (Rky. Mt. Herb.); rocky slopes, Mule Mountains, Bisbee, April, 1909, *Goodding 74* (Rky. Mt. Herb.).

Mexico:

Coahuila and Nuevo Leon: Feb. to Oct., 1880, *Palmer 31* (U. S. Nat. Herb.).

Nuevo Leon: mountains west of Icamole, Feb. 3, 1907, *Safford 1261* (U. S. Nat. Herb.).

Coahuila: Agua Nueva, April 18, 1905, *Palmer 558* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); vicinity of Saltillo, May, 1898, *Palmer 1821½* (U. S. Nat. Herb.); Sierra de Parras, March, 1905, *Purpus 1025* (Baker Herb. at Pomona College, and Mo. Bot. Gard. Herb.); Sierra de la Paila, Oct., 1910, *Purpus 4926* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Oro, Aug. 18, 1903, *Rose & Painter 6430* (U. S. Nat. Herb.); La Ventura, Aug. 2–5, 1896, *Nelson 3919* (U. S. Nat. Herb.); Agua Nueva, Feb. 14, 1847, *Gregg 91* (Mo. Bot. Gard. Herb.); Buena-vista, Feb. 19, 1847, *Gregg 304* (Mo. Bot. Gard. Herb.).

Chihuahua: St. Diego, April 10, 1891, *Hartman 615* (U. S. Nat. Herb.); Santa Eulalia Mountains, March 29, 1885, *Pringle 176* (U. S. Nat. Herb.).

Zacatecas: near Concepcion del Oro, Aug. 11–14, 1904, *Palmer 277* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); Hacienda de Cedros, 1908, *Lloyd 236* (U. S. Nat. Herb.).

*L. Fendleri* is certainly the most polymorphic species in this genus and yet there seem to be no natural lines of cleavage along which the species may be separated even varietally. Some strikingly different forms occur and it is possible to sort any considerable number of specimens into several distinguishable groups. If these groups are examined, however, the repre-

sentatives will be seen to have come from no particular part of the range of the species and several forms may be found from the same locality. This has been interpreted to mean that the forms are but ecological variants or represent minor races that occur independently again and again within the taxonomic species. One of the most marked of these forms has been given the specific name of *praecox*. This was first named the variety *δ. humilis* by Gray and is characterized by its low habit of growth, the shortened inflorescence, and the long-pedicelled flowers which are usually exceeded by the leaves. Hartman's collection from Chihuahua is the most aberrant specimen seen, and if future collections of this form are made from the same locality it would seem worthy of varietal rank. In this plant the pedicels are slightly sigmoid and the flowers red. The stellae, however, are quite normal for the species.

The most useful character in determining this species is found in the stellae. The entire plant has a silvery appearance due to the crowded scale-like or scurfy indument. Only *L. Schaffneri* and *L. pueblensis* of the glabrous podded forms have similar stellae and by careful comparison these can be differentiated by the form of the stellae alone. The chief differences between them lie in the pedicels, however. *L. Fendleri* seems to be without close relatives. Because of the unbranched rays of the scales it seems to be allied to the *argyraea-Schaffneri* group.

16. *L. Schaffneri* Wats. Proc. Am. Acad. 23: 254. 1888.

*Vesicaria Schaffneri* Wats. Proc. Am. Acad. 17: 320. 1882.

Perennial, silvery stellate throughout, rays numerous, mostly simple, united nearly or quite to their apices and under a lens appearing as small circular scales; stems simple or branched, erect or decumbent, 1-4 dm. long; terminal bud apparently developing into a normal, fertile stem; radical leaves narrowly oblanceolate, entire, repand or lyrate-pinnatifid, 2-6 cm. long, narrowed to a slender petiole; cauline leaves broadly oblanceolate to nearly linear, entire, repand or with a few prominent teeth, obtuse or acute, 1-3 cm. long, narrowed to a slender petiole; petals narrowly spatulate, about 7 mm. long, yellow, fading purplish (?) ; filaments slightly broadened at the base; fruiting inflorescence elongated; pedicels distinctly sigmoid, 7-10 mm.

long; pods erect, substipitate, glabrous, subglobose or oblong, 3-4 mm. in diameter; styles 2-3 mm. long; septum nerved, areolae rectangular, boundaries frequently tortuous; ovules 6-8 in each cell, funiculi attached to septum for about one-half their lengths; seeds small, marginless.

Distribution: in the mountains of north central Mexico.

Specimens examined:

Mexico:

Coahuila and Nuevo Leon: without definite locality, Feb.-Oct., 1880, *Palmer 29* (U. S. Nat. Herb.).

Coahuila: Sierra de Parras, March to April, 1905, *Purpus 1148* (Baker Herb. at Pomona College, and Mo. Bot. Gard. Herb.).

San Luis Potosi: chiefly in the region of San Luis Potosi, 1878, *Parry & Palmer 26* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); chiefly in the region of San Luis Potosi, 1878, *Parry & Palmer 25½* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); San Luis Potosi, 1879, *Schaffner 555* (U. S. Nat. Herb.); Minas de San Rafael, June, 1911, *Purpus 5232* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

This characteristic Mexican species is probably related most closely to *L. argyrea* and may be distinguished from all other members of the genus by the scale-like stellae in which the rays are attached nearly or quite to their apices.

#### 17. *L. pueblensis* Payson.<sup>1</sup>

Perennial, caudex usually branching and often elongated and woody; stellae numerous, giving the entire plant a silvery appearance, rays unbranched, united for about one-half their lengths, somewhat granular; stems mostly erect, 1-3.5 dm. long, slender, frequently branched; terminal bud apparently usually developing only a short sterile shoot; radical leaves unknown; cauline leaves narrowly oblanceolate, obtuse or acute, entire or repandly dentate, 2-4 cm. long; flowers small, inconspicuous; petals apparently at first yellow, fading purplish, broadly spatulate, about 6 mm. long; filaments linear, point of

<sup>1</sup>*Lesquerella pueblensis* sp. nov., subherbacea vel distincte fruticosa; foliis radicalibus ignotis; foliis caulis oblanceolatis, integris vel repando-dentatis, lepidostellatis; racemis frutiferis elongatis; pedicellis recurvatis; siliculis globosis, glabris, estipitatis, circiter 4 mm. diametro; loculis 2-3-ovulatis; stylis 1-2 mm. longis; funiculis septo adnatis; seminibus immarginatis.—Type collected in the vicinity of San Luis Tultitlanapa, Puebla, Mexico, by C. A. *Purpus 3389* (Mo. Bot. Gard. Herb.).

attachment slightly enlarged; fruiting inflorescence elongated, pedicels recurved, less than 1 cm. long; pods pendent, sessile, glabrous, globose, about 4 mm. in diameter; styles 1-2 mm. long; septum rather dense, nerved, areolae not tortuous; ovules 2-3 in each cell, funiculi short, attached to septum for about one-half their lengths; seeds not strongly flattened nor winged.

Distribution: State of Puebla, Mexico.

Specimens examined:

Mexico:

Puebla: collected in the vicinity of San Luis Tultitlanapa, near Oaxaca, July, 1908, *Purpus* 3389 (Mo. Bot. Gard. Herb., TYPE, and U. S. Nat. Herb.); near Tehuacan, Aug. 30-Sept. 8, 1905, *Rose, Painter & Rose* 10027 (U. S. Nat. Herb.).

Fig. 13. *L. pueblensis*. Habit sketch  $\times \frac{1}{2}$ . Trichomes  $\times 25$ .

This species is quite similar in appearance to *L. Schaffneri* and a casual comparison might fail to separate them. *L. Schaffneri* normally seems to develop a rosette, while the other plant does not; the rays of the stellae in *L. pueblensis* are free for about half their lengths, but in the related plant they are united nearly or quite to their apices; in *L. Schaffneri* the pedicels are usually sigmoid and the pods erect, in *L. pueblensis* the pedicels are recurved and the pods pendent. The ranges of the two species, as far as collections made up to the present time show, are well separated. *L. pueblensis*, so far as now known, is the southernmost North American species of the genus.

18. *L. recurvata* (Engelm.) Wats. Proc. Am. Acad. 23: 253. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 18. 1891; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 119. 1895; Heller, Contr. Herb. Franklin & Marshall College 1: 40. 1895; Small, Fl. Southeastern U. S. 470. 1903, ed. 2, 470. 1913.

*Vesicaria angustifolia* vars.  $\gamma$ . *longistyla integrifolia* and  $\delta$ . *longistyla pinnatifida* Scheele, *Linnaea* 21: 584. 1848; Roemer, Texas, 436. 1849.

*V. recurvata* Engelm. ex. Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 147. 1850; Walp. Ann. 2: 38. 1851.

*Alyssum recurvatum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Slender annuals, sparsely stellate; stellae scarcely contiguous, small, rays distinct, variable as to number and mode of branching; stems many, branching, erect or decumbent, 1–3.5 dm. long; terminal bud giving rise to a fertile stem; radical leaves thin, 2–4 cm. long, entire, shallowly toothed or rarely nearly lyrate, oblanceolate, narrowed to a slender petiole; cauline leaves entire, oblanceolate to nearly linear; petals yellow, narrowly spatulate, 5–6 mm. long; filaments linear, not dilated at the base; fruiting inflorescence elongated; pedicels very slender, recurved, 1 cm. or less long; pods pendent, sessile or nearly so, glabrous, globose, 2–4 mm. in diameter; styles slender, about as long as the pods; septum thin, nerved about half its length, areolae somewhat tortuous; ovules usually 5 in each cell, funiculi attached to septum for one-half their lengths or less.

Distribution: across central Texas from north to south.

Specimens examined:

Texas: dry soil, west of Crosstimbers, Johnson County, April, 1882, *Reverchon* (Mo. Bot. Gard. Herb.); light soil, Somerville County, April, *Reverchon* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); rocky soils, Falls Creek, Hood County, April, 1885, *Reverchon* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); Waco, *Pace 55* (Mo. Bot. Gard. Herb.); cultivated soil, Georgetown, Jan., 1890, *Bodin 63* (U. S. Nat. Herb.); rocky hill, Austin, May 20, 1872, *Hall 20* (U. S. Nat. Herb. and Mo. Bot.



Fig. 14. *L. recurvata*. Habit sketch  $\times \frac{1}{8}$ . Trichomes  $\times 25$ .

to base, areolae not tortuous; ovules 2 in each cell, funiculi attached to septum for about only one-fourth their lengths.

Distribution: in the mountains of southern New Mexico.

Specimens examined:

New Mexico: White Mountains, Lincoln County, July 30, 1897, *Wooton* 245 (Rky. Mt. Herb. and Mo. Bot. Gard. Herb.); Tularosa Creek, Lincoln County, Aug. 18, 1899, *Wooton* (U. S. Nat. Herb.); Cloudcroft, Aug. 24, 1901, *Wooton* (U. S. Nat. Herb. and Rky. Mt. Herb.); vicinity of Cloudcroft, Aug. 8, 1899, *Wooton* (U. S. Nat. Herb.); Sacramento Mountains, Aug. 7, 1905, *Wooton* (U. S. Nat. Herb.); Luna, July 28, 1900, *Wooton* (U. S. Nat. Herb.); White Mountains, Lincoln County, Aug. 18, 1899, *Wooton* (Deam Herb.).

*L. aurea* in technical characters does not differ greatly from *L. recurvata* but is quite a different plant in general appearance due to its coarse, weedy habit of growth. The number of ovules is reduced to 2, the basal leaves apparently are not so frequently, if at all, pinnate, and the pods instead of being definitely globose are often elongated, rather thin-walled, and irregularly inflated. Specimens from the same locality show glabrous and stellate pods on different plants. *Wooton* and *Standley* record it as occurring in the transition zone.

21. *L. argentea* (Pursh) MacMillan, Metasp. Minn. Valley, 263. 1892; Rydb. Contr. U. S. Nat. Herb. 3: 150. 1895; Britton & Brown, Ill. Fl. 2: 137. 1897, and ed. 2, 2: 155. 1913; Rydb. Mem. N. Y. Bot. Gard. 1: 179. 1900; Rydb. Fl. Colo. 155. 1906; Robinson & Fernald in Gray, Manual, ed. 7, 424. 1908; Nelson in Coulter & Nelson, Manual Cent. Rocky Mountains, 218. 1909; Gleason, Bull. Ill. State Lab. Nat. Hist. 9: 48. 1910; Petersen, Fl. Nebr. 62. 1912; Clements & Clements, Rocky Mountain Flowers, 25, 1914. Bergman, Fl. North Dakota, 191. 1918.

*Myagrum argenteum* Pursh, Fl. Am. Sept. 2: 434. 1816.

*Alyssum ludovicianum* Nutt. Gen. N. Am. Pl. 2: 63. 1818.

*Vesicaria ludoviciana* DC. Syst. 2: 297. 1821; Torr. & Gray, Fl. N. Am. 1: 101. 1838; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 149. 1850; Porter & Coulter, Syn. Fl. Colo. 7. 1874;

Macoun, Cat. Canadian Pl. 1: 54. 1883; Coulter, Manual Rocky Mountain Region, 25. 1885; Kellerman, Fl. Kan. 24. 1888.

*Lesquerella ludoviciana* (Nutt.) Wats. Proc. Am. Acad. 23: 252. 1888; Webber, Cat. Fl. Nebr. 119. 1890; Eastwood, Fl. Denver, 6. 1893; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 118. 1895; Nelson, Wyo. Exp. Sta. Bull. 28: 82. 1896; Rydb. Fl. Rocky Mountains, 333. 1917.

*Alyssum globosum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Stout perennial, densely stellate-pubescent throughout; stellae rather large, many-rayed, rays forking near the base, frequently granular; stems erect or decumbent, 2-4 dm. long, usually unbranched; terminal bud remaining undeveloped or central stem elongating slightly but always sterile; radical leaves broadly linear to narrowly oblanceolate, 3-10 cm. long, entire or slightly toothed; cauline leaves similar but shorter, numerous; petals yellow, very narrow, blade and claw scarcely distinct, about 8 mm. long; filaments slightly but gradually dilated at the base; fruiting inflorescence elongated; pedicels recurved, 1-1.5 cm. long; pods usually pendent, sessile or substipitate, globose or slightly elongated, 3-4 mm. in diameter, densely pubescent with more or less spreading stellae; styles slender, equalling or exceeding the pods; septum thin, distinctly nerved, areolae straight or somewhat tortuous; ovules 4-6 in each cell, funiculi attached to septum for about one-half their lengths.

Distribution: Illinois, Minnesota, North and South Dakota, Montana, southern Wyoming, Colorado, eastern Utah, and northern Arizona.

Specimens examined:

Illinois: on sand dunes, Havana, Aug. 22, 1904, *Gleason* (Deam Herb.).

North Dakota: sandy soil on hillside, Cannon Ball, June 28, 1912, *Bergman* 1875 (Mo. Bot. Gard. Herb.); Mandan, 1915, *Sarvis* 6 (U. S. Nat. Herb.).

South Dakota: common at Fort Pierre, June 19, 1853, *Hayden* (Mo. Bot. Gard. Herb.); Eaglenest Butte, on White River, May 14, 1855, *Hayden* (Mo. Bot. Gard. Herb.); bed of Cheyenne River, July 3, 1859, *Hayden* 88 (Mo. Bot. Gard. Herb.);



gravelly places on Eaglenest Butte, Washabaugh County, May 30, 1914, *Over 2005* (U. S. Nat. Herb.); Edgemont, Fall River County, June 2, 1897, *Stanton* (Mo. Bot. Gard. Herb.); Crook, Harding County, July 23, 1910, *Visher 176* (Rky. Mt. Herb.).

Nebraska: Cheyenne County, June 8, 1901, *Baker* (Mo. Bot. Gard. Herb.); Long Pine, June 1, 1899, *Bates* (Rky. Mt. Herb.); Wiegand, July 8, 1893, *Clements 2693* (U. S. Nat. Herb.); Sidney, May 14, 1914, *Eggleston 9030* (U. S. Nat. Herb.); Hershey, May 12, 1903, *Mell* (U. S. Nat. Herb.); on sand hills, Halsey, June 2, 1903, *Mell & Knopf* (Mo. Bot. Gard. Herb.); Long Pine, May 20, 1893, *Rutter* (U. S. Nat. Herb.); hills, Deuel County, June 25, 1891, *Rydberg* (U. S. Nat. Herb.); sand hill, Middle Loup River near Thedford, Thomas County, June 17, 1893, *Rydberg 1281* (U. S. Nat. Herb.); Pine Ridge, June 27, 1899, *Webber* (Mo. Bot. Gard. Herb.); Belmont, July 25, 1889, *Webber* (Mo. Bot. Gard. Herb.); Pine Ridge, June 18, 1890, *Williams 333* (U. S. Nat. Herb.); Platte River, June 18, 1890, *Williams 46* (Rky. Mt. Herb.); Belmont, June 17, 1890, *Williams* (U. S. Nat. Herb.).

Kansas: gravelly soil, Logan County, May 9, 1895, *Hitchcock 16* (Mo. Bot. Gard. Herb., U. S. Nat. Herb., and Rky. Mt. Herb.).

Montana: Great Falls, June 9, 1885, *Williams 5* (U. S. Nat. Herb.); mouth of Sand Coulee, May 28, 1885, *Anderson* (U. S. Nat. Herb.).

Wyoming: plateau east of Cheyenne, June 25, 1880, *Engelmann* (Mo. Bot. Gard. Herb.); gravelly hills near Red Buttes, May 10, 1860, *Hayden* (Mo. Bot. Gard. Herb.); Tie Siding, June 26, 1919, *Osterhout 5914* (Geo. Osterhout Herb.); sterile hills, Yellowstone, 1853-4, *Hayden* (Mo. Bot. Gard. Herb.); Lance Creek, July 4, 1896, *Knowlton 134* (U. S. Nat. Herb.); Laramie, June 17, 1895, *Nelson 1310* (Mo. Bot. Gard. Herb., U. S. Nat. Herb., and Rky. Mt. Herb.); university campus, Laramie, June, 1898, *Nelson 59* (U. S. Nat. Herb.); Laramie, June 1, 1894, *Nelson 190* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); Pratt, Laramie County, June 27, 1901, *Nelson 8284* (Mo. Bot. Gard. Herb., U. S. Nat. Herb., and Rky. Mt. Herb.);

common on the plains, Laramie, June 1, 1894, *Nelson 3949* (Rky. Mt. Herb.); open dry flats, Laramie, June 20, 1900, *Nelson 7275* (Rky. Mt. Herb., Mo. Bot. Gard. Herb., Geo. Osterhout Herb., and U. S. Nat. Herb.); Laramie River, June 15, 1891, *Buffum 61* (Rky. Mt. Herb.); railroad grade, Rock River, June 18, 1901, *Goodding 26* (Rky. Mt. Herb.); Fort Steele, Carbon County, May 25–June 10, 1901, *Tweedy 4489* (U. S. Nat. Herb.); T. B. Ranch, Carbon County, June 20, 1901, *Goodding 56* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); dry soil near Washington Ranch, June 30, 1901, *Merrill & Wilcox 733* (U. S. Nat. Herb. and Rky. Mt. Herb.); dry soil 35 miles north of Point of Rocks, June 21, 1901, *Merrill & Wilcox 521* (Rky. Mt. Herb.); Fort Bridger, July, 1873, *Porter* (U. S. Nat. Herb.); Carter, June 25, 1896, *Jones* (U. S. Nat. Herb., Mo. Bot. Gard. Herb., and Rky. Mt. Herb.).

Colorado: Clear Creek, June, 1873, *Wolfe* (U. S. Nat. Herb.); Rocky Mountains, lat. 40–41°, 1868, *Vasey 47* (Mo. Bot. Gard. Herb.); Livermore, Larimer County, July 25, 1904, *Osterhout 2864* (Geo. Osterhout Herb.); Livermore, Larimer County, May 27, 1919, *Osterhout 5888* (Geo. Osterhout Herb.); Sterling, Logan County, June 10, 1896, *Osterhout 1103* (Geo. Osterhout Herb.); Windsor, Weld County, June 6, 1896, *Osterhout 1102* (Geo. Osterhout Herb.); Hugo, June 12, 1907, *Marsh* (U. S. Nat. Herb.); Crow Creek, June 26, 1896, *Knowlton 94* (U. S. Nat. Herb.); Evans, June 1, 1908, *Johnston 164* (Mo. Bot. Gard. Herb.); lat. 41°, 1862, *Hall & Harbour 48* (Mo. Bot. Gard. Herb.); Denver, July, 1884, *Ball* (U. S. Nat. Herb.); north of Craig, Routt County, June 10, 1902, *Osterhout 2621* (Geo. Osterhout Herb.); upper juniper area, west of Delta, June 6, 1909, *Tidesvrom 2176* (U. S. Nat. Herb.).

Utah: Pahria Canyon, May 26, 1894, *Jones 5297a* (U. S. Nat. Herb.).

Arizona: 1869, *Palmer* (U. S. Nat. Herb.).

This species has a most interesting geographic distribution. East of the mountains from Montana to south-central Colorado it is a common plant, as it also is in southern Wyoming. Its southern extension to Arizona, however, is marked by very few collections. These collections are quite typical and evidently

the species does occur rarely west of the Continental Divide. It would seem that the comparatively low break in the north and south line of the Rockies that occurs across southern Wyoming made possible the migration of this species far from its point of origin.

The record from Illinois by Gleason is the most remarkable extension of range known for any species of *Lesquerella*. The single plant collected is fragmentary and imperfect owing to the season being so far advanced at the time that the collection was made. Collections made early in the summer may show characters not possessed by this specimen, but at present there seems no reason to regard it other than typical *argentea*. The plants occur "in considerable numbers" on sand dunes about twelve miles northeast of Havana.

From *L. recurvata* and *L. aurea*, this species is separated by its perennial habit of growth and its densely stellate pods. From *L. macrocarpa* and *L. purpurea*, with which it may also be confused because of the recurved pedicels, it is distinguished by the narrow basal leaves. A few specimens have been seen in which the pedicels were straight and ascending but this is evidently a rare variation.

22. *L. arenosa* (Richards.) Rydb. Bull. Torr. Bot. Club 29: 236. 1902; Rydb. Fl. Rocky Mountains, 333. 1917; Bergman, Fl. North Dakota, 191. 1918.

*Vesicaria arenosa* Richards. Franklin's Journey to the Shores of the Polar Sea, 743. 1823; DC. Prodr. 1: 160. 1824.

*V. arctica* Hook. Bot. Mag. 3: t. 2882. 1829.

*V. arctica* var.  $\beta$ . Torr. & Gray, Fl. N. Am. 1: 100. 1838; Hook. Fl. Bor. Am. 1: 48. 1840.

*V. ludoviciana* Macoun, Cat. Canadian Pl. 1: 54. 1883.

*Lesquerella ludoviciana* (Nutt.) Wats. var. *arenosa* Wats. Proc. Am. Acad. 23: 252. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 118. 1895.

*L. argentea arenosa* Rydb. Contr. U. S. Nat. Herb. 3: 485. 1896.

*L. versicolor* Greene, Pittonia 4: 310. 1901; Rydb. Fl. Rocky Mountains, 333. 1917.

*L. Macounii* Greene, Pittonia 4: 310. 1901; Rydb. Fl. Rocky Mountains, 333. 1917.

*L. rosea* Greene, Pittonia 4: 311. 1901; Rydb. Fl. Rocky Mountains, 333. 1917.

*L. Lunellii* Nelson, Bot. Gaz. 42: 49. 1906.

*L. Lunellii* var. *lutea* Nelson, Bot. Gaz. 54: 149. 1912.

Perennial, stellate pubescent throughout, rays long, usually forked near base, irregular; stems slender, decumbent, occasionally branching, .5–2.5 dm. long, terminal bud remaining undeveloped; radical leaves usually rather thin, 1–6 cm. long, blade narrowly oblanceolate to oval, entire or remotely dentate, usually narrowed into a slender petiole; cauline leaves linear to oblanceolate, entire, .5–4 cm. long; petals narrow, about 7 mm. long, yellow or variously tinged with red or purple; stamens filiform; fruiting inflorescence elongated; pedicels slender, recurved or horizontal, 1 cm. or less long; pods globose or slightly elongated, pendent or nearly horizontal, sessile, stellate-pubescent, about 3 mm. in diameter; styles slender, 3–5 mm. long; septum thin, nerved, areolae somewhat tortuous; ovules 4–7 in each cell, funiculi attached to septum for about half their lengths; seeds small, not margined.

Distribution: southern Manitoba, Saskatchewan and Alberta, North and South Dakota, and probably eastern Montana.

Specimens examined:

Manitoba: Stony Mountain, June 4, 1896, *Macoun 12401* (U. S. Nat. Herb.).

Saskatchewan: bluffs, Moose Jaw, June 22, 1907, *Cowles 62* (Mo. Bot. Gard. Herb.).

• Alberta: Medicine Hat, June 1, 1894, *Macoun* (U. S. Nat. Herb.); dry hills, Elbow River Valley, vicinity of Calgary, April 25, 1915, *Moodie 810* (U. S. Nat. Herb.).



Fig. 15. *L. arenosa*. Habit sketch  $\times \frac{1}{3}$ . Trichomes  $\times 25$ .

North Dakota: on gravelly, hilly plains, Dunseith, Rolette County, June 3, 1911, *Lunell* (Rky. Mt. Herb.); stony barren summits of hills, Butte, Benson County, May 14–June 4, 1905, *Lunell* (U. S. Nat. Herb. and Rky. Mt. Herb.); Leeds, Benson County, June 5, 1909, *Lunell* (Rky. Mt. Herb.); Leeds, Benson County, May 28, 1901, *Lunell* (Rky. Mt. Herb.); railroad banks, Leeds, June 10, 1899, *Lunell* (U. S. Nat. Herb.); dry hills, Butte, Benson County, May 20–June 10, 1906, *Lunell* (Geo. Osterhout Herb., Deam Herb., U. S. Nat. Herb., and Rky. Mt. Herb.); dry hills, Towner, McHenry County, May 29, 1908, *Lunell* (Geo. Osterhout Herb., U. S. Nat. Herb., Deam Herb., and Rky. Mt. Herb.); on gravelly hills, Minot, Ward County, June 5 and 6, 1909, *Lunell* (Rky. Mt. Herb.); on sunny slopes, Williston, May 2, 1906, *Lunell* (Rky. Mt. Herb.); Dickinson, June 30, 1912, *Waldron 128* (Deam Herb.).

South Dakota: Date, Perkins County, June 15, 1912, *Visher 571* (Rky. Mt. Herb.); slopes of Cedar Pass, Stanley County, June 6, 1914, *Over 6268* (U. S. Nat. Herb.); prairie, Newell, May 12, 1913, *Carr 8* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); Hot Springs, June 11, 1892, *Rydberg 533* (U. S. Nat. Herb.).

*L. arenosa* is likely to be confused with no other species except *L. argentea*, and from that it is distinguished chiefly by the very slender stems and the much smaller size. The leaves in *arenosa* are broader in proportion to their length and more frequently toothed than those of *argentea*. The pubescence in the smaller species is less dense than in the larger one. Finally, the ranges of the two, although apparently overlapping to some extent, are in general rather definitely separated. It may be found impossible to retain *L. arenosa* in specific rank and the treatment accorded it by Watson may be ultimately reinstated. No true intermediates have been seen.

The phylogenetic position of this species with relation to *L. argentea* is not clear. The characters separating the two are slight but *L. arenosa* resembles *L. recurvata*, the parental type of this group, more than does *L. argentea*. The geographical position of the two, however, would indicate that *L. arenosa* is derived from *L. argentea* rather than the reverse. Several gregates have been proposed but the differences they represent

seem no more than the variations between individuals. *L. versicolor* is a form in which the pedicels are not recurved and the flowers are tinged with red. *L. rosea* has recurved pedicels and red flowers. Bergman, from field observation, has noticed the color of the flowers in this species to be exceedingly variable.

23. *L. macrocarpa* A. Nelson, Bot. Gaz. 34: 366. 1902; Nelson in Coulter & Nelson, Manual Cent. Rocky Mountains, 218. 1909; Rydb. Fl. Rocky Mountains, 333. 1917.

Perennial, finely stellate-pubescent throughout, rays distinct, branched; stems decumbent or procumbent, .5–2 dm. long, simple or branched, rather stout; terminal bud remaining undeveloped or producing a short, sterile shoot; radical leaves 1–4 cm. long, blade ovate or orbicular, entire or sparingly toothed, abruptly narrowed to a petiole; cauline leaves numerous, ovate to oblanceolate, obtuse, entire, 1–3 cm. long; petals yellow, broadly spatulate, about 7 mm. long; filaments linear; fruiting inflorescence elongated; pedicels stout, recurved, usually less than 1 cm. long; pods pendent, sessile, stellate-pubescent, when fully developed often 6–7 mm. in diameter, valves rather thin and irregularly inflated; styles 1.5–2 mm. long; septum reduced to a narrow margin around the replum, areolae tortuous; ovules 2–4 in each cell, funiculi attached to septum; seeds flattened, not margined, large.

Distribution: known as yet only from Sweetwater County in southwestern Wyoming.

Specimens examined:

Wyoming: naked clay flats and ridges, Bush Branch, Sweetwater County, June 10, 1900, *A. Nelson 7081* (Rky. Mt. Herb., TYPE, Mo. Bot. Gard. Herb., and Geo. Osterhout Herb.); dry soil, 45 miles north of Point of Rocks, June 21, 1900, *Merrill & Wilcox 568* (U. S. Nat. Herb. and Rky. Mt. Herb.).

This is a quite distinct species apparently of restricted range, having its nearest relative in *L. argentea*. From that species it is amply distinct by the broader leaves, the procumbent instead of erect stems, and the fewer number of ovules in the cells. In all the specimens examined the septum is nearly obsolete, being reduced to a narrow margin within the replum. Bush Ranch is not far from Steamboat Mountain, near the base of which the type was collected.

24. *L. angustifolia* (Nutt.) Wats. Proc. Am. Acad. 23: 253. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 120. 1895; Small, Fl. Southeastern U. S. 471. 1903, and ed. 2, 471. 1913.

*Vesicaria angustifolia* Nutt. ex Torr. & Gray, Fl. N. Am. 1: 101. 1838; Walp. Rep. 1: 141. 1842; Dietr. Gen. Pl. 3: 639. 1843.

Biennial ?, canescent with scarcely contiguous stellae, stellae small, rays few to many, branched; stems numerous, slender, erect or decumbent, 1–2 dm. high, branched; terminal bud apparently developing into a fertile stem; radical leaves 1–2 cm. long, entire to sublyrate, narrowly oblanceolate, narrowed to a slender petiole; cauline leaves linear to narrowly oblanceolate,



Fig. 16. *L. angustifolia*. Habit sketch  $\times \frac{1}{2}$ . Trichomes  $\times 25$ .

entire, 1–2 cm. long; petals yellow, spatulate, about 5 mm. long; filaments abruptly dilated at the base; fruiting inflorescence short but not crowded; pedicels straight, strongly ascending, slender, 7–8 mm. long; pods erect, subsessile, glabrous, exactly globose, 2–3 mm. in diameter; styles slender, 3–4 mm. long; septum thin, nerved, areolae tortuous; ovules 2 in each cell, funiculi attached to septum less than one-half their lengths; seeds flat, not

margined.

Distribution: Arkansas, southwestern Missouri, and probably western Oklahoma.

Specimens examined:

Missouri: Willard, 1887, *Blankinship* (U. S. Nat. Herb.); Greene County, April 30, 1887, *Blankinship* (Mo. Bot. Gard. Herb.); Greene County, June, 1899, *Plank* (Mo. Bot. Gard. Herb.); Springfield, May 7, 1887, *Blankinship* (Mo. Bot. Gard. Herb.).

From *L. gracilis*, with which this species has been confused in herbaria, it is at once separable by the numerous slender stems that apparently never attain the height reached by *L. gracilis*. The sessile pods, the dilated bases of the filaments, and the few ovules in each cell serve farther to distinguish these

two species. *L. angustifolia* is probably limited to calcareous soils.

25. *L. Lindheimeri* (Gray) Wats. Proc. Am. Acad. 23: 253. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 18. 1891; Wats. in Gray, Syn. Fl. N. Am. 1: 120. 1895; Small, Fl. Southeastern U. S. 470. 1903, ed. 2, 470. 1913.

*Vesicaria Lindheimeri* Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 145. 1850; Walp. Ann. 2: 39. 1851.

*Alyssum Lindheimeri* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

*Lesquerella Gordonii* Heller, Contr. Herb. Franklin and Marshall College 1: 40. 1895.

Annual or short-lived perennial, canescent throughout with minute stellae, rays few to many, branched, distinct or irregularly coherent; stems erect or decumbent, 1.5–4 dm. long, simple or branched; terminal bud apparently developing a fertile stem; radical leaves usually more densely stellate beneath, lyrate-pinnatifid, oblanceolate, 2–6 cm. long; cauline leaves lanceolate or oblanceolate, conspicuously toothed, 1–3 cm. long; petals yellow, spatulate; filaments slightly enlarged at the base; fruiting inflorescence elongated; pedicels horizontal, more or less sigmoid, 8–13 mm. long; pods erect or ascending, glabrous, subglobose, 4–5 mm. in diameter, stipe 1 mm. or more long; septum nerved, areolae not tortuous; ovules about 8 in each cell, funiculi attached to the septum for about one-half their lengths; seeds not margined nor winged.

Distribution: southern Texas.

Specimens examined:

Texas: black, stiff prairie soil east of Victoria, Feb., 1845, *Lindheimer* 327 (Mo. Bot. Gard. Herb.); along Corpus Christi

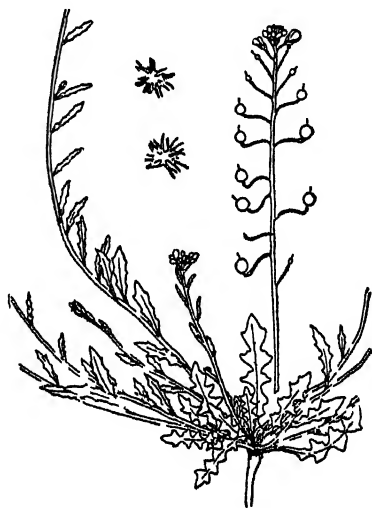


Fig. 17. *L. Lindheimeri*. Habitsketch  $\times \frac{1}{2}$ . Trichomes  $\times 25$ .



Bay, Nueces County, March 21, 1894, *Heller 1478* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

*L. Lindheimeri*, from an examination of the type collection, seems unquestionably different from any other species of this genus. The failure of other collectors in this same region to get this species again, however, makes its position rather doubtful. The collection by Heller from Corpus Christi is scarcely typical but nearer the species than to *L. Gordonii* and so helps somewhat to confirm *L. Lindheimeri*. The present species is likely to be confused with *L. gracilis* or *L. Gordonii*. From the former the horizontally spreading and sigmoid pedicels separate it and from the latter it may be distinguished by the longer stipe and the more conspicuously pinnatifid leaves. In the type collection the stellae are remote on the upper surface of the leaves and closely overlapping on the lower. They resemble those of *L. gracilis* much more than those of *L. Gordonii*.

26. *L. gracilis* (Hook.) Wats. Proc. Am. Acad. 23: 253. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 18. 1891; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 119. 1895; Robinson & Fernald in Gray, Manual, ed. 7, 424. 1908; Britton & Brown, Ill. Fl. 2: 137. 1897, and ed. 2, 2: 155. 1913.

*Vesicaria gracilis* Hook. Bot. Mag. N. S. 10: t. 3533. 1836; Walp. Rep. 1: 141. 1842; Dietr. Syn. Pl. 3: 638. 1843; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 148. 1850; Walp. Ann. 2: 38. 1851.

*V. polyantha* Schlecht. Bot. Zeit. 11: 619. 1853.

*Alyssum gracile* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

*Lesquerella polyantha* Small, Fl. Southeastern U. S. 471. 1903, and ed. 2, 471. 1913.

Annual, pubescence stellate, stellae often scarcely contiguous, small, rays numerous, distinct or irregularly coherent; stems many, slender, erect or decumbent, 3-5 dm. long, often branching; terminal bud developing a fertile stem; radical leaves narrowly oblanceolate, 3-8 cm. long, from nearly entire to coarsely lyrate; cauline leaves from linear and entire to oblanceolate and repandly dentate, narrowed at the base, 1-4.5 cm. long; petals yellow, broadly spatulate, about 7 mm. long; filaments linear,

not dilated at the base; fruiting inflorescence elongated, open; pedicels usually straight and ascending, slender, 1–2 cm. long; pods erect or ascending, glabrous, globose to ellipsoid, 3–4 mm. in diameter, borne on a slender stipe 1–2 mm. long; styles slender, 2–3 mm. long, stigma capitate; septum nerved, areolae straight or slightly tortuous; ovules 8–10 in each cell, funiculi attached for about one-half their lengths; seeds not margined, flat.

Distribution: from north to south across central Oklahoma and Texas.

Specimens examined:

Missouri: on the railway bank, Glen Allen, Bollinger County, May, 1900, *Russell* (Mo. Bot. Gard. Herb.).

Oklahoma: Stillwater, April 12, 1897, *Bogue* (U. S. Nat. Herb.).

Texas: Brazos Santiagos, 1889, *Nealley 148* (U. S. Nat. Herb.); fields and waste ground, Dallas, May 19, 1903, *Biltmore Herb. 2693a* (U. S. Nat. Herb.); rich prairies, Dallas, April 14, 1902, *Reverchon 2970* (Mo. Bot. Gard. Herb.); cement works, Dallas County, March 25, 1902, *Reverchon* (Mo. Bot. Gard. Herb.); calcareous soil, Dallas, March 18, 1900, *Reverchon* (Mo. Bot. Gard. Herb.); creek near Dallas, May 3, 1900, *Eggert* (Mo. Bot. Gard. Herb.); light soil, Dallas, April, 1880, *Reverchon 40* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Viewpoint on Ft. Worth & Cleburne Interurban, April 9, 1913, *Ruth 39* (Mo. Bot. Gard. Herb.); prairie near Mustang Creek, Tarrant County, May 11, 1900, *Eggert* (Mo. Bot. Gard. Herb.); Navarro County, 1880, *Joor 93* (U. S. Nat. Herb.); Limestone County, 1878, *Joor 95* (U. S. Nat. Herb.); Waco, *Pace 44* (Mo. Bot. Gard. Herb.); Austin, March 17, 1908, *York 385* (Mo. Bot. Gard. Herb.); dry prairies, Austin, May 18, 1872, *Hall 22* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); common on prairie, Columbia, April 18, 1899, *Bush 186* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); New Braunfels, April, 1850, *Lindheimer 668* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Muskit Flats, New Braunfels, March, 1846, *Lindheimer 331* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Muskit Flats, New Braunfels, May, 1845, *Lindheimer 299* (Mo. Bot. Gard. Herb.); Robstown, Nueces County, April 11, 1905, *Lewton 118* (U. S. Nat. Herb.); Corpus Christi,

March 31, 1905, *Tracy 9196* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Typical *L. gracilis* is characterized by the nearly globose pods borne on long, slender stipes. It is a rather polymorphic and weedy plant and is evidently common in fields and waste places throughout its range. On the north it passes gradually into the variety *repanda*. The one specimen cited for Missouri is typical *gracilis* and represents probably an introduction from farther south, since it was collected upon a railroad embankment and has not been confirmed by other collections from this state. The species and its varieties occur, at least in part, upon calcareous soils.

26a. *Var. repanda* (Nutt.) Payson, new comb.

*Vesicaria repanda* Nutt. in Torr. & Gray, *Fl. N. Am.* 1: 101. 1838; Walp. *Rep.* 1: 141. 1842; Dietr. *Syn. Pl.* 3: 639. 1843; Gray, *Bost. Jour. Nat. Hist. (Pl. Lindh.)* 6: 148. 1850; Walp. *Ann.* 2: 38. 1851.

*Vesicaria Nuttallii* Torr. & Gray, *Fl. N. Am.* 1: 101. 1838; Walp. *Rep.* 1: 141. 1842; Dietr. *Syn. Pl.* 3: 639. 1843; Gray, *Bost. Jour. Nat. Hist. (Pl. Lindh.)* 6: 148. 1850; Walp. *Ann.* 2: 38. 1851.

*Lesquerella repanda* Wats. *Proc. Am. Acad.* 23: 252. 1888; Wats. in Gray, *Syn. Fl. N. Am.* 1<sup>a</sup>: 119. 1895; Small, *Fl. Southeastern U. S.* 470. 1903, and ed. 2, 470. 1913.

*L. Nuttallii* Wats. *Proc. Am. Acad.* 23: 252. 1888; Wats. in Gray, *Syn. Fl. N. Am.* 1<sup>a</sup>: 119. 1895; Small, *Fl. Southeastern U. S.* 470. 1903, and ed. 2, 470. 1913.

*Alyssum repandum* Kuntze, *Rev. Gen. Pl.* 2: 931. 1891.

*A. Nuttallii* Kuntze, *Rev. Gen. Pl.* 2: 931. 1891.

*Lesquerella gracilis* Petersen, *Fl. Nebraska*, 62. 1912.

Annual; stems branching; radical leaves sinuate-dentate to lyrate; cauline leaves numerous, repandly dentate; petals yellow; fruiting inflorescence elongated; pedicels horizontal to ascending; pods erect, glabrous, obpyriform, frequently with a distinct shoulder near the base, 5–6 mm. long, borne on a slender stipe 1–2 mm. long; ovules 5–8 in each cell.

Distribution: northeastern Arkansas, southeastern Kansas, Oklahoma, and north central Texas.

## Specimens examined:

Arkansas: in the Cherokee country, between the Illinois and Neosho rivers, June, 1835, *Engelmann* 781 (Mo. Bot. Gard. Herb.).

Kansas: vicinity of Oswego, May 11, 1883, *Oyster* (Mo. Bot. Gard. Herb.); Oswego, May 5, 1891, *Newton* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); low rocky prairie near Independence, April 26, 1902, *Kenoyer* (U. S. Nat. Herb.).

Oklahoma: Goodland, May 10, 1892, *Trelease* (Mo. Bot. Gard. Herb.); common, Catoosa, May 8, 1895, *Bush* 1152 (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); grassy prairie, Marietta, Love County, April 18, 1913, *Stevens* 88 (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Texas: sandy soil, Terrell, April 5, 1903, *Reverchon* 3716 (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

The only consistent difference between this plant and *gracilis* lies in the pear-shaped rather than spherical pods. All intermediates between the two are found. In the specimens cited those plants having globose or ellipsoid pods have been referred to the species and those in which the pods were decidedly pyriform have been retained for the variety. *L. repanda* was described from a plant with immature pods, while in the type of *L. Nuttallii* the pods were fully developed. The shoulder at the base and the broadly pear-shaped apex do not develop until the pod is nearly mature.

26b. Var. *sessilis* Wats. Proc. Am. Acad. 23: 253. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 18. 1891; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 120. 1895.

*Lesquerella sessilis* Small, Fl. Southeastern U. S. 471. 1903, and ed. 2, 471. 1913.

*Vesicaria angustifolia* Scheele, Linnaea 21: 584. 1848; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 145. 1850; Gray, Smithsonian. Contr. (Pl. Wright.) 5: 13. 1853.

*V. gracilis* Torr. & Gray, Pac. R. R. Rept. 2: 159. 1855.

Annual; stems branching; radical leaves entire to lyrate; cauline leaves linear to oblanceolate, entire or repandly dentate; petals yellow; fruiting inflorescence elongated; pedicels ascend-

ing; pods ascending or erect, sessile, glabrous, globose or slightly elongated, 3–4 mm. in diameter.

Distribution: central to western Texas.

Specimens examined:

Texas: 1851, *Wright 848* (U. S. Nat. Herb.); Llano Valley, 1888, *Reverchon 42* (Mo. Bot. Gard. Herb.); Grape Creek, Gillespie County, *Jerry* (Mo. Bot. Gard. Herb.); calcareous open ground, Boerne, Kendall County, April 21, 1917, *Palmer 11622* (Mo. Bot. Gard. Herb.); summits of hills, New Braunfels, March, 1846, *Lindheimer 326* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); New Braunfels, May, 1850, *Lindheimer 301* (Mo. Bot. Gard. Herb.); New Braunfels, May, 1850, *Lindheimer 669* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Bexar County, June, 1904, *Jerry* (Mo. Bot. Gard. Herb.); rocky soils, San Antonio, March 18, 1903, *Reverchon* (Mo. Bot. Gard. Herb.); light soil, Mackenzie's Well, Crockett County, May, 1888, *Reverchon 42* (U. S. Nat. Herb.).

This variety occurs with the species throughout its range, and there seems no reason to believe that it is anything more than an occasional variation from the typical form since it differs from the species in but one character—the absence of a stipe to the pod.

27. *L. Gordonii* (Gray) Wats. Proc. Am. Acad. 23: 253. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 18. 1895; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 120. 1895; Wootton & Standley, Contr. U. S. Nat. Herb. 19: 275. 1915; Armstrong, Western Wild Flowers, 184. 1915; Rydberg, Fl. Rocky Mountains, 333. 1917.

*Vesicaria Gordonii* Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 149. 1850; Walp. Ann. 2: 38. 1851.

*Alyssum Gordonii* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Annual, canescent with long-rayed, overlapping stellae, rays distinct, forked at the base; stems 1–3.5 cm. long, decumbent, few to many, branched in the older, more vigorous plants; terminal bud producing either a normal stem or frequently a short stem floriferous nearly to the base; radical leaves 1.5–3.5 cm. long, narrowly oblanceolate or spatulate, acute, tapering to a slender petiole, entire or somewhat repand, rarely lyrate with

two basal lobes; cauline leaves 1–3 cm. long, numerous, linear or narrowly oblanceolate, narrowed at the base, entire or slightly repand; petals yellow, narrowly obovate, claw slightly broadened at the base; filaments linear; fruiting inflorescence elongated; pedicels rather stout, horizontal or somewhat recurved, distinctly sigmoid, about 1 cm. long; pods usually erect, often horizontal, globose, glabrous, about 4 mm. in diameter, stipe evident but less than 1 mm. long; style about 3 mm. long; septum thin, nerved, areolae slightly tortuous; ovules 4–10 in each cell, funiculi long, attached to septum for about one-third their lengths; seeds flat, not margined.

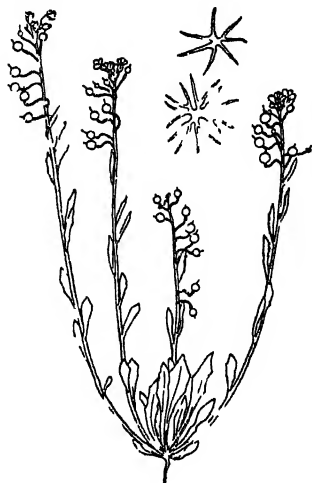


Fig. 18. *L. Gordonii*. Habit sketch  $\times \frac{1}{2}$ . Trichomes  $\times 25$ .]

Distribution: western Oklahoma and Texas, southern New Mexico, southeastern Arizona, northern Chihuahua, and Sonora.

Specimens examined:

Oklahoma: Glass Mountains, July 13, 1899, *White 140* (Mo. Bot. Gard. Herb.); Cimarron Valley, Cherokee Outlet, June, 1891, *Carleton 214* (U. S. Nat. Herb.); prairies near Woodward, June 5, 1901, *Eggert* (Mo. Bot. Gard. Herb.).

Texas: west of Cross Timbers, April, 1882, *Reverchon* (Mo. Bot. Gard. Herb.); light soil, Brown County, April, 1882, *Reverchon* (U. S. Nat. Herb.); sandy prairies near Comanche, Comanche County, May 10, 1900, *Eggert* (Mo. Bot. Gard. Herb.); Knickerbocker Ranch, Dove Creek, Tom Greene County, May, 1880, *Tweedy* (U. S. Nat. Herb.); San Angelo, May 19, 1903, *Reverchon* (Mo. Bot. Gard. Herb.); plains west of Pecos, April 20, 1902, *Tracy & Earle 119* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.); sandy ground near Van Horn, May 13, 1901, *Eggert* (Mo. Bot. Gard. Herb.); Rio Grande, 60 miles below El Paso, March, 1852, *Wright 1318* (Mo. Bot. Gard. Herb.); sandy ground near Sierra Blanca, El Paso County, May 13, 1901; *Eggert* (Mo. Bot. Gard. Herb.); Big Springs,

May 11, 1902, *Tracy 8043* (U. S. Nat. Herb.); rocky prairies near Colorado, Mitchell County, June 10, 1900, *Eggert* (Mo. Bot. Gard. Herb.); Grady, Fisher County, March 31, 1901, *Shepherd* (U. S. Nat. Herb.); sandy plains, Estelline, May 24-25, 1904, *Reverchon 4288* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); sandy soil, Amarillo Creek, May 30, 1902, *Reverchon* (Mo. Bot. Gard. Herb.); dry prairies near Canadian, Hemphill County, June 7, 1901, *Eggert* (Mo. Bot. Gard. Herb.).

New Mexico: *Parry, Bigelow, Wright & Schott 44* (U. S. Nat. Herb.); on the upper Canadian in the mountains of New Mexico, April, 1848, *Gordon* (Mo. Bot. Gard. Herb.); Gray, Lincoln County, 1898, *Skehan* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Mesilla Valley, March 31, 1907, *Wooton & Standley* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); near Mesilla, May 4, 1906, *Standley* (U. S. Nat. Herb.); Mesilla Valley, April 26, 1905, *Wooton* (U. S. Nat. Herb. and Deam Herb.); Mesilla Valley, April 24, 1893, *Wooton* (U. S. Nat. Herb.); Lake Valley, April, 1914, *Beals* (U. S. Nat. Herb.); near Silver City, June 2, 1880, *Greene* (Mo. Bot. Gard. Herb.); Mangas Springs, Grant County, April 20, 1903, *Metcalf 23* (Rky. Mt. Herb., U. S. Nat. Herb., and Mo. Bot. Gard. Herb.); Mangas, May, 1897, *Metcalf 48* (U. S. Nat. Herb.); Mangas Springs, Grant County, April 3, 1880, *Rusby 14* (Mo. Bot. Gard. Herb.); Mangas Springs, Sept. 3, 1880, *Rusby 398* (U. S. Nat. Herb.).

Arizona: Andrade, March 13-April 23, 1903, *Griffiths 4091* (U. S. Nat. Herb.); Skull Valley, June 4, 1865, *Coues & Palmer 237* (Mo. Bot. Gard. Herb.); Skull Valley, May 3, 1865, *Coues & Palmer 188* (Mo. Bot. Gard. Herb.); on mesas, Camp Grant, March 10, 1867, *Palmer 10* (Mo. Bot. Gard. Herb.); grassy knolls, common, May 3, 1865, *Coues & Palmer 197* (Mo. Bot. Gard. Herb.); Santa Rosa to Casa Grande, March 13-April 23, 1903, *Griffiths 4011* (U. S. Nat. Herb.); Canaca to Arabaca, March 13-April 23, 1903, *Griffiths 3548* (U. S. Nat. Herb.); vicinity of Duncan, April, 1908, *Rose 11740* (U. S. Nat. Herb.); Santa Catalina Mountains, March, 1881, *Vasey* (U. S. Nat. Herb.); Tucson, 1911, *Beard* (Mo. Bot. Gard. Herb.); hills near Tucson, April, 1880, *Pringle* (U. S. Nat. Herb.); Tumamoc Hill, Tucson, Feb. 28, 1914, *Harris C. 142* (U. S. Nat. Herb.); small range reserve near Tucson, March 13-April 23, 1903,

*Griffiths 3531* (U. S. Nat. Herb.); Tucson, Feb. 20, 1892, *Toumey 66* (U. S. Nat. Herb.); Tucson, March 20, 1894, *Toumey* (U. S. Nat. Herb.); Tucson Mountains, March 13–April 23, 1903, *Griffiths 3493* (U. S. Nat. Herb.); campus, Univ. of Arizona, Tucson, March 14, 1903, *Thornber 369* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Santa Rita Range Reserve, May 19, 1912, *Wooton* (U. S. Nat. Herb.); Santa Rita Forest Reserve, March 31–April 23, 1903, *Griffiths 3905* (U. S. Nat. Herb.); Arivapa Canyon, March, 1873, *Mohr* (U. S. Nat. Herb.).

Mexico:

Chihuahua (?): Carrizallito Mountains, April 19, 1892, *Mearns 5* (U. S. Nat. Herb.).

*L. Gordonii* is a common plant throughout at least a large part of its range and occurs associated with other cruciferous weeds in waste places. Its distinguishing characteristics are the annual root, the sigmoid pedicels, and the shortly stipitate, globose, glabrous pod. Its nearest relative seems to be *L. Palmeri* with which it may be found to merge in Arizona.

28. *L. Palmeri* Wats. Proc. Am. Acad. 23: 255. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 118. 1895.

*L. Gordonii* (Gray) Wats. var. *sessilis* Wats. Proc. Am. Acad. 23: 253. 1888; Coulter, Contr. U. S. Nat. Herb. 2: 18. 1891; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 120. 1895.

*L. tenella* A. Nels. Bot. Gaz. 47: 426. 1909.

Annual, stellae rather small, frequently sparse, rays numerous, forked at base, distinct, finely granular; stems slender, decumbent or erect, 1–4 dm. long, in the larger plants usually branched; terminal bud developing a normal, fertile stem; radical leaves entire to lyrate with few lobes, 1–5 cm. long, narrowed to a slender petiole; cauline leaves linear to oblanceolate, entire or slightly repand, 1–5 cm. long; petals yellow, broadly spat-



Fig. 19. *L. Palmeri*. Habit sketch  $\times \frac{1}{2}$ . Trichomes  $\times 25$ .



ulate, claw somewhat broadened at the base; filaments linear; fruiting inflorescence elongated; pedicels ascending, horizontal or recurved, usually sigmoid, 1 cm. or less long; pods erect, horizontal or, rarely, nearly pendent, sessile, or substipitate, sparsely stellate-pubescent, globose or slightly elongated, 3-5 mm. in diameter; styles 2-3 mm. long; septum nerved, areolae somewhat tortuous; ovules 4-6 in each cell, funiculi attached to septum for about one-half their lengths; seeds flattened, not winged.

Distribution: southern Utah, western Arizona, southeastern Nevada, California, and northern Lower California.

Specimens examined:

Utah: southern Utah, 1875, *Johnson* (U. S. Nat. Herb.).

Arizona: Beaverdam, April 5, 1894, *Jones 5024e* (U. S. Nat. Herb.); mesa north of Phoenix, Feb. 9, 1912, *Wooton* (U. S. Nat. Herb.); Hassayampa Valley, April 12, 1876, *Palmer 570* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Yucca, March 12, 1912, *Wooton* (U. S. Nat. Herb.); Yucca, May 13, 1884, *Jones 3879* (U. S. Nat. Herb.).

Nevada: at base of cliffs, Meadow Valley Wash, April 6, 1905, *Goodding 2155* (Rky. Mt. Herb.); Bunkerville, April 6, 1894, *Jones 5029b* (U. S. Nat. Herb.); among the undershrub, Moapa, April 8, 1905, *Goodding 2184* (Rky. Mt. Herb. and Mo. Bot. Gard. Herb.); Moapa, Lincoln County, May 12, 1905, *Kennedy 1096* (U. S. Nat. Herb.); Vegas Wash, Lincoln County, near its junction with the Colorado River, March 11, 1891, *Coville & Funston 406* (U. S. Nat. Herb.).

California: Canyon Springs, Riverside County, April 22, 1905, *Hall 5845* (Rky. Mt. Herb.); Salvation Springs, Riverside County, April 24, 1905, *Hall 5882* (U. S. Nat. Herb.).

Mexico:

Lower California: Topo Canyon, July 9, 1884, *Orcutt 1099* (Mo. Bot. Gard. Herb.).

*L. Palmeri* has usually been confused with *Gordonii* from which it is separated by its sparsely stellate, rather than glabrous, and sessile or subsessile, rather than stipitate, pods. It is usually a more slender plant than *L. Gordonii* and the stems seem less inclined to branch. The pedicels are at times recurved and so suggest the *recurvata* group but they nearly always show a noticeable tendency to become S-shaped. This species is

apparently able to survive in a more truly desert region than any other member of the genus. The plants complete their growth and mature their seeds very early in the season.

29. *L. pinetorum* Wooton & Standley, Contr. U. S. Nat. Herb. 16: 126. 1913; Wooton & Standley, Contr. U. S. Nat. Herb. 19: 276. 1915.

Perennial, densely stellate throughout, stellae rather small, rays numerous, branched, distinct or somewhat coherent, granular; stems decumbent or erect, 1–2.5 dm. long, simple; terminal bud usually developing a short fertile stem; radical leaves oblanceolate, spatulate or nearly oval, entire or irregularly denticulate, 2–6 cm. long, narrowed to a slender petiole; cauline leaves spatulate to oblanceolate, usually obtuse, entire or denticulate, 1–4 cm. long; petals yellow, narrowly spatulate, 7–10 mm. long; filaments linear; fruiting inflorescence elongated; pedicels distinctly sigmoid, 8–10 mm. long; pods usually erect, glabrous, sessile, globose, 3–5 mm. in diameter; styles 3–5 mm. long; septum nerved, areolae not tortuous; ovules 5–7 in each cell, funiculi attached to septum for less than one-half their lengths; seeds large, flat, not winged.

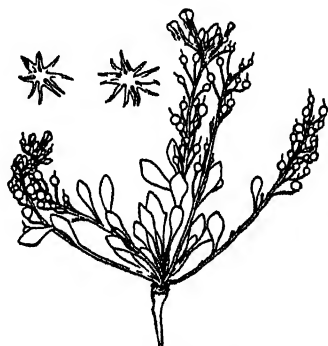


Fig. 20. *L. pinetorum*. Habit sketch  $\times \frac{1}{6}$ . Trichomes  $\times 25$ .

Distribution: central New Mexico.

Specimens examined:

New Mexico: Sandia Mountains, June, 1898, *Herrick 204* (U. S. Nat. Herb.); dry ridges, Balsam Park, Sandia Mountains, April 7, 1911, *Ellis 7* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); San Mateo Mountains, March 27, 1895, *Herrick 531* (U. S. Nat. Herb.); Kingston, Sierra County, May 2, 1905, *Metcalf 1534* (Mo. Bot. Gard. Herb.); White Mountain Peak, Aug. 16, 1897, *Wooton* (U. S. Nat. Herb.); vicinity of Gilmore's Ranch, on Eagle Creek, Lincoln County, July 29, 1901, *Wooton* (U. S. Nat. Herb.); White Mountains, Lincoln County, Aug. 25, 1907, *Wooton & Standley 3460* (U. S. Nat. Herb., TYPE).

To *L. pinetorum* is referred a somewhat heterogeneous group

of specimens characterized by glabrous, nearly globose pods, conspicuously sigmoid pedicels, and an imperfect rosette. As to size of the pods, form of the basal leaves, and leafiness of the stem there is considerable variation. The series of specimens at hand is scarcely enough to show whether or not the species should be broken up into two or three varieties, and accordingly the present treatment is regarded as rather provisional. The type is characterized by small pods and numerous stem-leaves.

30. *L. pruinosa* Greene, *Pittonia* 4: 307. 1901.

Perennial, canescently stellate-pubescent throughout, stellae

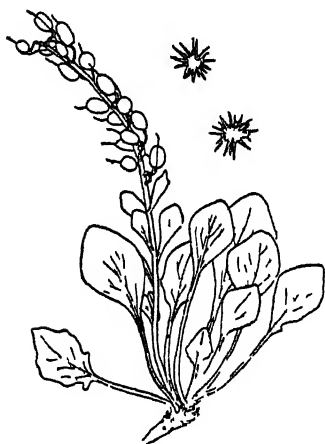


Fig. 21. *L. pruinosa*. Habit sketch  $\times \frac{1}{4}$ . Trichomes  $\times 25$ .

small, scarcely contiguous on fully developed leaves, many-rayed, rays forked, irregularly coherent; caudex woody, sometimes branched; stems decumbent or erect, 10–17 cm. long, unbranched; terminal bud remaining undeveloped; radical leaves 3–8 cm. long, blade indefinitely quadrate to oval, entire or repand, obtuse, abruptly narrowed to the slender petiole which exceeds it in length, petiole frequently purplish, pruinose with not closely contiguous stellae; cauline leaves obovate, entire or few-toothed, obtuse, 1–2 cm. long; flowers sulphur-yellow, small; fruiting inflorescence elongated, rather crowded;

pedicels conspicuously sigmoid, 5–6 mm. long; pods erect, sessile or subsessile, glabrous, ellipsoid, 6–9 mm. long; styles slender, 4–6 mm. long; septum nerved, entire, areolae conspicuously tortuous; ovules 3–4 in each cell, funiculi attached to the septum for less than one-half their lengths; seeds not margined.

Distribution: southern Colorado.

Specimens examined:

Colorado: Pagosa Springs, Archuleta County, July 4, 1917, Bethel (Geo. Osterhout Herb. and Bethel Herb.).

This species is most closely related to *L. pinetorum* and marks a decided advance in specialization as well as a considerable

step in the northward progression of this line of development. It is definitely separated from *pinetorum* by its conspicuous rosette and broad-bladed radical leaves.

Although the type of *L. pruinosa* has not been seen there seems no doubt that Prof. Bethel's collection from the type locality is specifically identical with it. The only point of difference in Dr. Greene's description and the specimen seen is in the length of the stem. The type is described as having "peduncles and short racemes not greatly surpassing the foliage even in fruit." In Prof. Bethel's plant the fruiting inflorescence is carried well above the tuft of radical leaves.

31. *L. lata* Wooton & Standley, Contr. U. S. Nat. Herb. 16: 126. 1913; Wooton & Standley, Contr. U. S. Nat. Herb. 19: 275. 1915.

Perennial, densely stellate throughout, stellae rather small, rays numerous, branching, granular; stems erect or spreading, about 1 dm. long, simple; terminal bud (in type specimen) giving rise to a very short stem, floriferous to the base; radical leaves 3-4 cm. long, blade entire, obtuse, broadly oval, narrowed to a long slender petiole; cauline leaves broadly oblanceolate, entire, obtuse, 1-2 cm. long; petals yellow, narrowly spatulate, about 7 mm. long; filaments linear; fruiting inflorescence elongated or slightly compacted; pedicels sigmoid, 5-7 mm. long; pods usually erect, sessile, sparsely stellate-pubescent, globose or somewhat obpyriform, 1.5-3 mm. in diameter; styles slender, 3-4 mm. long; septum nerved, areolae not tortuous; ovules 5-6 in each cell, funiculi attached to septum about one-fourth their lengths; seeds not winged:



Fig. 22. *L. lata*. Habit sketch  $\times \frac{1}{8}$ . Trichomes  $\times 25$ .

Distribution: in the White Mountains of southern New Mexico.

Specimens examined:

New Mexico: collected in or near the Lincoln National Forest, 1903, *Plummer* (U. S. Nat. Herb., TYPE); White Mountain Peak, alt. 9600 feet, July 6, 1895, *Wooton* (U. S. Nat. Herb.).

*L. lata*, although known by very few collections, may well stand as a distinct step between the glabrous and stellate podded species of this group. The sparsely pubescent pods distinguish it from *L. pinetorum*, while the broad stem-leaves and the imperfect rosette separate it from *L. rectipes*. In the type specimen the terminal stem is greatly shortened and bears pods nearly to the base. This intermediate step between the species that show no inhibition of the terminal bud and those forming a perfect rosette is most interesting. This character is shared likewise by *L. pinetorum*.

32. *L. rectipes* Wooton & Standley, Contr. U. S. Nat. Herb. 16: 127. 1913; Wooton & Standley, Contr. U. S. Nat. Herb. 19: 217. 1915.

*L. montana* Rydb. Fl. Colo. 155. 1906, in part.



Fig. 23. *L. rectipes*. Habit sketch  $\times \frac{1}{2}$ . Trichomes  $\times 25$ .

Perennial, densely stellate throughout, stellae many-rayed, rays branched, distinct or somewhat coherent, granular; stems decumbent, 1-4 dm. long, usually simple; terminal bud remaining undeveloped; radical leaves from narrowly oblanceolate to ovate, entire or repandly dentate, 2-5 cm. long, narrowed to a slender petiole; cauline leaves linear to narrowly oblanceolate, usually quite entire, 1-3 cm. long; petals yellow, narrowly oblanceolate, about 7 mm. long; filaments linear; fruiting inflorescence frequently showing a tendency to remain crowded near the summit of the stem; pedicels in age usually sigmoid, 8-10 mm. long; pods horizontal to erect, sessile, sparsely stellate-pubescent, globose or slightly elongated, 3-5 mm. in diameter; styles 3-5 mm. long; septum nerved, areolae scarcely tortuous; ovules 3-6 in each cell, funiculi attached to septum for about one-half their lengths; seeds flattened, neither winged nor margined.

Distribution: southwestern Colorado, northwestern New Mexico, southeastern Utah, and northeastern Arizona.

Specimens examined:

Colorado: Mancos, Montezuma County, June 15, 1899, *Osterhout 1947* (Geo. Osterhout Herb.); Mancos, June 24, 1898, *Baker, Earle & Tracy 85* (Rky. Mt. Herb., Geo. Osterhout Herb., Mo. Bot. Gard. Herb., Baker Herb. at Pomona College, and U. S. Nat. Herb.); Los Pinos, May, 1899, *Baker 254* (Baker Herb. at Pomona College, U. S. Nat. Herb., Rky. Mt. Herb., Geo. Osterhout Herb., and Mo. Bot. Gard. Herb.); dry valley lands, Paradox, Montrose County, June 21, 1912, *Walker 150* (Rky. Mt. Herb.); rocky slopes, Paradox, June 22, 1912, *Walker 168* (Rky. Mt. Herb.); dry, stony slopes of hills east of Montrose, June 15, 1915, *Payson 669* (Rky. Mt. Herb.); dry hills, Naturita, May 11, 1914, *Payson 294* (Rky. Mt. Herb. and Mo. Bot. Gard. Herb.).

New Mexico: northwestern New Mexico, June 6, 1883, *Marsh 81* (U. S. Nat. Herb., TYPE); 13 miles south of Atarque de Garcia, July 19, 1906, *Wooton* (U. S. Nat. Herb.); between Salt Lake and Atarque de Garcia, July 19, 1906, *Wooton* (U. S. Nat. Herb.); Navajo Indian Reservation, in the Tunitcha Mountains, Aug. 8, 1911, *Sandley 7787* (U. S. Nat. Herb.); along the Rio Grande, west of Santa Fe, May 31, 1897, *Heller 3634* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Utah: western slope of La Sal Mountains, near Little Springs, July 5-6, 1911, *Rydb. & Garrett 8558* (Rky. Mt. Herb. and U. S. Nat. Herb.); Armstrong and White Canyons, near the Natural Bridges, Aug. 4-6, 1911, *Rydb. & Garrett 9448* (U. S. Nat. Herb. and Rky. Mt. Herb.).

This species is most likely to be confused with *L. montana* and is distinguished from it chiefly by the normally globose pods. The ranges of the two nearly meet in New Mexico but farther north the Continental Divide separates them. The pods in *L. montana* are densely stellate, while the stellae on those of *L. rectipes* are rarely contiguous.

33. *L. montana* (Gray) Wats. Proc. Am. Acad. 23: 251. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 117. 1895; Rydb. Fl. Colo. 155. 1906; Nelson in Coulter & Nelson, Manual Cent. Rocky

Mountains, 219. 1909; Nelson, Spring Fl. Intermountain States, 65. 1912; Clements & Clements, Rocky Mountain Flowers, 25. 1914; Wooton & Standley, Contr. U. S. Nat. Herb. 19: 275. 1915; Rydb. Fl. Rocky Mountains, 332. 1917.

*Vesicaria montana* Gray, Proc. Acad. Nat. Sci. Phila. 1863: 58, 1863; Porter & Coulter, Syn. Fl. Colo. 7. 1874; Coulter, Manual Rocky Mountain Region, 25. 1885.

*Alyssum Grayanum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

*Lesquerella rosulata* Nelson, Bull. Torr. Bot. Club 25: 205. 1898.

*L. Shearis* Rydb. Bull. Torr. Bot. Club 29: 237. 1902; Rydb. Fl. Colo. 155. 1906; Daniels, Univ. Mo. Studies 2: 128. 1911; Rydb. Fl. Rocky Mountains, 332. 1917.

*L. curvipes* Rydb. Fl. Colo. 155. 1906; Rydb. Fl. Rocky Mountains, 332. 1917, in part.

Perennial, cinerous stellate-pubescent throughout, stellae few to many-rayed, rays forked near the base, distinct or irregularly coherent; caudex frequently unbranched, rarely enlarged; stems decumbent, 1-2 dm. long, unbranched or rarely branched; terminal bud remaining undeveloped; radical leaves quite variable in outline, 1.5-4 cm. long, entire, narrowly oblanceolate, acute with blade tapering gradually to the petiole or blade ovate to oblong, abruptly narrowed to the slender petiole, entire or toothed, frequently obtuse; cauline leaves from very narrowly oblanceolate to broadly cuneate and then frequently with 2 conspicuous lateral teeth, acute or obtuse, 1-3 cm. long, numerous; petals yellow, narrowly spatulate, 7-9 mm. long; filaments linear, slightly enlarged at point of attachment; fruiting inflorescence elongated; pedicels conspicuously sigmoid, 8-12 mm. long; pods erect, sessile, densely stellate-pubescent, oblong, 6-8 mm. long, obtuse or acute but not conspicuously compressed at the apex; styles 3-6 mm. long; septum conspicuously nerved, areolae straight or tortuous; ovules 6-10 in each cell, funiculi attached to the septum for less than one-half their lengths; seeds not margined.

Distribution: southwestern South Dakota, southeastern Wyoming, eastern Colorado and northeastern New Mexico.

Specimens examined:

South Dakota: Hot Springs, June 6, 1893, *Schneck* (Mo. Bot. Gard. Herb.).

Wyoming: open, sandy plains, Cheyenne, May, 1902, *Nelson 8843* (Mo. Bot. Gard. Herb., Rky. Mt. Herb., and U. S. Nat. Herb.); Cheyenne, June 14, 1916, *Eggleston 12552* (U. S. Nat. Herb.); near Table Mountain, June 2, 1894, *Nelson 3757* (Rky. Mt. Herb.); Table Mountain, June 2, 1895, *Nelson 88* (U. S. Nat. Herb.); Dixon Canyon, May 27, 1890, *Buffum 60* (Rky. Mt. Herb.); Pole Creek, June 29, 1895, *Nelson 1370* (Mo. Bot. Gard. Herb., Rky. Mt. Herb., and U. S. Nat. Herb.); Centennial Valley, Aug. 18, 1896, *Nelson* (Rky. Mt. Herb.); Laramie River, June 29, 1900, *E. Nelson 265* (Rky. Mt. Herb.); stony ridges, Laramie Hills, June 5, 1900, *Nelson 7256* (Mo. Bot. Gard. Herb. and U. S. Nat. Herb.).

Colorado: without definite locality, 1871, *Greene* (Mo. Bot. Gard. Herb.); New Windsor, June, 1895, *Osterhout 4426* (Geo. Osterhout Herb.); New Windsor, June 2, 1908, *Osterhout 3851* (Geo. Osterhout Herb.); Spring Canyon, Larimer County, May, 1895, *Osterhout 785* (Geo. Osterhout Herb.); Fossil Creek, Larimer County, June 25, 1917, *Osterhout 5615* (Geo. Osterhout Herb.); Owl Canyon, Larimer County, May 27, 1919, *Osterhout 5889* (Geo. Osterhout Herb.); Ft. Collins, May 27, 1894, *Baker* (Mo. Bot. Gard. Herb.); Ft. Collins, May 15, 1895, *Baker* (Rky. Mt. Herb.); Ft. Collins, May 15, 1896, *Baker* (Mo. Bot. Gard. Herb.); Ft. Collins, May 19, 1897, *Crandall 212* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Long Canyon and Rist Canyon, May 31, 1896, *Baker & Holzinger 92* (U. S. Nat. Herb.); Estes Park, July, 1884, *Ball* (U. S. Nat. Herb.); Estes Park, July 1, 1912, *Churchill* (Mo. Bot. Gard. Herb.); North Park, 1881, *Broadhead 105* (Mo. Bot. Gard. Herb.); Michigan Creek, North Park, Jackson County, July 31, 1913, *Osterhout 4993* (Geo. Osterhout Herb.); near Boulder, June 3, 1901, *Ramaley 710* (Rky. Mt. Herb.); near Boulder, July, 1902, *Tweedy 5067* (Rky. Mt. Herb.); near Boulder, May 30, 1905, *Ramaley 1027* (Rky. Mt. Herb.); St. Vrain Creek, June 9, 1906, *Dodds 1889* (Rky. Mt. Herb.); Lyons, in foothills, May 24, 1916, *Johnston 850* (U. S. Nat. Herb.); plains near Denver, May 8, 1900, *Rydberg & Vreeland 6137* (Geo. Osterhout Herb.); Clear Creek, June-July, 1873, *Wolfe* (U. S. Nat. Herb.); Middle Mountains, 39-41° lat., 1862, *Hall & Harbour 49* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); plains, Colorado Springs, May 4, 1878,



*Jones 19* (U. S. Nat. Herb.); dry, gravelly soil on hills near Colorado Springs, June 18, 1896, *Biltmore Herb. 2695a* (U. S. Nat. Herb.); east of Garden of Gods, June 22, 1896, *Biltmore Herb. 1292* (U. S. Nat. Herb.); Colorado Springs, May 8, 1897, *Heller 3509* (Mo. Bot. Gard. Herb.); mesas near Colorado Springs, May 9, 1900, *Rydborg & Vreeland 6145* (Rky. Mt. Herb.); vicinity of Colorado Springs, June 19–30, 1915, *Eggleston 11195* (U. S. Nat. Herb.); Canyon City, April 1, 1871, *Brandege 25* (Mo. Bot. Gard. Herb.); South Park, 1873, *Wolfe 641* (U. S. Nat. Herb.); Arthur's, South Park, June 2, 1910, *Eggleston 5632* (U. S. Nat. Herb.); Salida, June 19, 1898, *Baker, Earle & Tracy 901* (Baker Herb. at Pomona College, and Mo. Bot. Gard. Herb.); Salida, June 27, 1917, *Payson 1017* (Mo. Bot. Gard. Herb.); river bluffs, north of La Veta, May 21, 1900, *Rydborg & Vreeland 6139* (Rky. Mt. Herb.); mountain near La Veta, June 20, 1900, *Rydborg & Vreeland 6141* (Rky. Mt. Herb.).

New Mexico: volcanic hills, on and near the Sierra Grande, Union County, June 18, 1911, *Standley 6054* (U. S. Nat. Herb.); Pecos River, June 6, 1897, *Heller* (U. S. Nat. Herb.).

*L. montana*, although quite a variable species as regards leaf outline, is usually at once distinguishable by the sigmoid pedicels and elongated pods. *L. rosulata* was described from an abnormal plant of a form with broad basal leaves. In Colorado *L. montana* crowds close upon the Continental Divide, but in the typical form seems never to have crossed it. Osterhout's No. 4993 is aberrant because of its shorter, slightly obcompressed pods. If further collections of it are made it would seem worthy of varietal rank.

### 33a. Var. *suffruticosa* Payson.<sup>1</sup>

Caudex enlarged and woody, branching in the older specimens; radical leaves silvery stellate-pubescent, 2–6 cm. long, oblanceolate, blade gradually narrowed to the slender petiole, irregularly dentate or repand, usually acute; pods oblong, 6–8 mm. long; styles 2–6 mm. long; ovules 7–10 in each cell.

Distribution: southern Colorado to northeastern New Mexico.

Specimens examined:

<sup>1</sup> *Lesquerella montana* (Gray) Wats. var. *suffruticosa*, var. nov., caudex amplius, suffruticosus; foliis radicalibus 2–6 cm. longis, oblanceolatis, inaequaliter dentatis vel repandis.—Collected on dry hills on or near the Sierra Grande, Union County, New Mexico, June 20, 1911, P. C. Standley 6249 (U. S. Nat. Herb.).

Colorado: south of Trinidad, Las Animas County, July 21, 1918, *Osterhout 5781* (Geo. Osterhout Herb.); Trinidad (road to Walsenburg), June 20, 1917, *Johnston 976* (Geo. Osterhout Herb.); Silverton, July 10, 1895, *Tweedy 147* (U. S. Nat. Herb.).

New Mexico: dry hills on and near the Sierra Grande, Union County, June 20, 1911, *Standley 6249* (U. S. Nat. Herb., TYPE); dry hills, vicinity of Raton, Colfax County, June 21 and 22, 1911, *Standley 6294* (U. S. Nat. Herb.).

This variety is characterized by an excessive development of the caudex and by the more silvery, larger basal leaves which are usually toothed. The specimen from Silverton, Colorado, is the only one seen from west of the Continental Divide and is nearly, if not quite, typical.

34. *L. curvipes* A. Nelson, Bull. Torr. Bot. Club 25: 205. 1898; Nelson in Coulter & Nelson, Manual Cent. Rocky Mountains, 219. 1909; Rydb. Fl. Rocky Mountains, 332. 1917, in part.

Perennial, stellate-pubescent throughout, stellae frequently rather remote, many-rayed, rays distinct or irregularly coherent, forked; stems erect or decumbent, 1-4 dm. long, often branched; terminal bud remaining undeveloped; radical leaves linear-ob lanceolate, the outermost sometimes oval, entire or repand, 3-6 cm. long, acute; cauline leaves linear-ob lanceolate, entire, 2.5-5 cm. long; petals narrowly spatulate, yellow; filaments linear; fruiting inflorescence elongated; pedicels conspicuously sigmoid, 8-15 mm. long; pods erect, sessile, stellate-pubescent, ovate or oblong, distinctly compressed at the apex, 6-8 mm. long; styles 2-4 mm. long; septum nerved, areolae more or less tortuous; ovules 4-6 in each cell, funiculi attached to the septum for about one-half their lengths; seeds not margined, radical turned slightly to one side.



Fig. 24. *L. curvipes*. Habits sketch  $\times \frac{1}{8}$ . Trichomes  $\times 25$ .

Distribution: northern Wyoming and southern Montana.

Specimens examined:

Montana: Red Lodge, July 26, 1893, *Rose 42* (U. S. Nat. Herb.).

Wyoming: Dome Lake Grade, July 18, 1896, *Nelson 2424* (Rky. Mt. Herb., TYPE); stony foothills west of Hurlbut Creek, June 15, 1909, *Willits 94* (Rky. Mt. Herb.); dry slope, hills southeast of Sheridan, June 15, 1913, *Sharp 339* (Rky. Mt. Herb.); Buffalo, July, 1900, *Tweedy 3588* (Rky. Mt. Herb.); from seed grown at Laramie, 1899, *Nelson & Nelson* (Rky. Mt. Herb. and Mo. Bot. Gard. Herb.).

*L. curvipes* is closely related to *L. montana* and may be distinguished from it by the pods which are strongly compressed at the apex. It is isolated geographically from *montana* since their ranges are not known to approach one another closely.

35. *L. globosa* (Desv.) Wats. Proc. Am. Acad. 23: 252. 1888; Wats. in Gray, Syn. Fl. N. Am. 1': 118. 1895; Britton & Brown, Ill. Fl. 2: 136. 1897, and ed. 2, 2: 154. 1913; Robinson & Fernald in Gray, Manual, ed. 7, 424. 1908; Small, Fl. South-eastern U. S. 470. 1903, and ed. 2, 470. 1913.

*Vesicaria globosa* Desv. Jour. Bot. 3: 184. 1814; Dietr. Syn. Pl. 3: 638. 1843.

*V. Shortii* Torr. & Gray, Fl. N. Am. 1: 102. 1838, supplement, 668. 1840; Walp. Rep. 1: 141. 1842; Dietr. Syn. Pl. 3: 639. 1843; Gray, Bost. Jour. Nat. Hist. (Pl. Lindh.) 6: 148. 1850.

*Alyssum Shortii* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

Biennial or perennial, canescent throughout with a rather loose stellate pubescence; stellae few- to many-rayed, rays forked; caudex somewhat woody, more or less elongated, unbranched; stems many, frequently branched, rather slender, 2.5-5 dm. long; terminal bud developing into a fertile stem; radical leaves 2.5-5 cm. long, oblong, entire or repand-toothed, blade gradually narrowed to the short petiole, strongly veined beneath; cauline leaves nearly linear to lanceolate, entire or repand, 1-4 cm. long, narrowed to a slender base; petals yellow, spatulate, 4-5 mm. long; filaments linear, slightly enlarged at the base; fruiting inflorescence elongated; pedicels slender, nearly straight,

horizontal or ascending, 7–11 mm. long; pods horizontal or ascending, sessile, sparsely stellate-pubescent or glabrous, globose, 1–2 mm. in diameter; styles filiform, 2–3 mm. long, stigma capitate; septum slightly nerved at the apex, areolae scarcely, if at all, tortuous; ovules 1–2 in each cell, funiculi attached to the septum for one-half their lengths or less; seeds nearly or quite filling the pods, not margined, radical somewhat turned to one side.

Distribution: Kentucky and northern Tennessee.

Specimens examined:

Kentucky: rocky soil, Valley View, May 16, 1903, *Biltmore Herb.* 4273a (U. S. Nat. Herb.); banks of Kentucky River, Frankfort, *Lesquereux* (Mo. Bot. Gard. Herb.).

Tennessee: limestone bluffs, Whites Bend, Davidson County, May 25, 1899, *Biltmore Herb.* 4273 (U. S. Nat. Herb.); Nashville, 1886, *Gattinger* (U. S. Nat. Herb.); Rising Sun Bluff, 14 miles below Nashville, April-May, 1886, *Gattinger* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

The original description of *Vesicaria globosa* Desv. is scarcely sufficient to confirm its specific identity with *V. Shortii* Gray, and this uncertainty is increased by reference to the 'Kew Index' and Kuntze's 'Revision' in which it is considered identical with *ludoviciana* (*argentea* MacM.). Fortunately, however, Dr. F. Gagnepain, of the Museum of Natural History in Paris, to whom fragments of *L. argentea* and *L. globosa* were sent, was able to confirm the present treatment by comparison with Desvaux's type. Dr. Gagnepain adds that the label on the type sheet reads *V. globulosa* instead of *V. globosa*.

*L. globosa* is apparently without close relatives in the genus and one is at a loss to know to which group of species to ally it. That its affinities are with this genus, however, there seems little doubt. The species may be readily recognized by the very numerous, small pods and the straight pedicels together with the conspicuously branching stems.

36. *L. mendocina* (Phil.) Kurtz, *Revista Mus. La Plata* (Sertum Cordobense) 5: 286. 1893; Macloskie, *Rep. Princeton Univ. Exp. to Patagonia* 8: 440. 1905.

*Vesicaria arctica* Hook. *Bot. Misc.* 3: 138. 1883; Barneoud

in Gay, Fl. Chilena 1: 161. 1845; Gilg & Muschler, Engl. Bot. Jahrb. 42: 466. 1909.

*V. mendocina* Phil. Linnaea 33: 12. 1864.

*V. andicola* Gill mss.; Ball, Jour. Linn. Soc. 21: 212. 1886.

*Alyssum Urbanianum* Muschler, Engl. Bot. Jahrb. 40: 274. 1908.

*A. bolivense* Muschler, Engl. Bot. Jahrb. 40: 275. 1908.

Perennial, densely stellate-pubescent throughout, stellae many-rayed, rays forked, coalescing at the base; stems decumbent or prostrate, 5–12 cm. long, unbranched; terminal bud remaining undeveloped; radical leaves 2–3 cm. long, narrowly oblanceolate, rarely over 5 mm. wide, entire or repand, very gradually narrowed to a slender petiole; cauline leaves narrowly oblanceolate, often rather numerous; petals yellow, obovate, about 1 cm. long; filaments linear; fruiting inflorescence rather short, lax; pedicels straight or often sigmoid, 1–2 cm. long; pods erect to horizontal, sessile, densely stellate-pubescent, short-ellipsoid, not compressed, 7–9 mm. long; styles about 4 mm. long, stigmas capitate; septum strongly nerved, areolae scarcely tortuous; ovules about 5 in each cell, funiculi long and slender, attached to the septum for about one-half their lengths.

Distribution: northern Argentine, adjacent Chile, and Bolivia.

Specimen examined:

Argentina: Cerro Negro, Catamarca, Sept. 2, 1916, *Jorgensen 1062* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Bolivia: Escayache bei Tarija, 3600 M., austro-Bolivia, Feb. 1, 1904, *K. Fiebig 3034* (Gray Herb.); Puna Patanca, 3700 M., Jan. 8, 1904, *K. Fiebig 2619* (Gray Herb.).

This species, although curiously isolated from its fellows, bears all the characteristics of a true member of this genus and seems more nearly related to certain species of the Rocky Mountains than to *L. montevidensis* of Uruguay. Kurtz observes that this plant grows in dry and especially in calcareous habitats. The flowers are said to be fragrant.

37. *L. valida* Greene, Pittonia 4: 68. 1899; Wooton & Standley, Contr. U. S. Nat. Herb. 19: 275. 1915.

Densely silvery stellate throughout; stems numerous, stout, decumbent, 12–15 cm. long, axillary to the outer leaves of a

rosulate tuft; radical leaves obovate or somewhat spatulate, entire or few-toothed, tapering to a petiole; cauline leaves oblanceolate, entire; inflorescence short and dense, hardly more than corymbose even in fruit; pods ovate, somewhat compressed, tipped with a style of half their own length; ovules about 6 in each cell.

Distribution: Gray, New Mexico.

The type of *L. valida* has not been seen nor have any specimens that could be referred to it. The ovate, compressed pod, as well as the short, dense inflorescence, seems to ally it to *L. intermedia*, with which, indeed, Wootton and Standley associate it. The plant is evidently a perennial although the original description leaves this in doubt. The root is described as a "single tap root." The type was collected at Gray, New Mexico, by Miss Josephine Skehan in 1898.

38. *L. intermedia* (Wats.) Heller, Plant World 1: 22, 1897; Wootton & Standley, Contr. U. S. Nat. Herb. 19: 275. 1915; Rydb. Fl. Rocky Mountains, 332. 1917.

*Vesicaria alpina* Gray, Mem. Am. Acad. (Pl. Fendl.) 4: 9. 1849, not Nutt.

*Lesquerella alpina* (Gray) Wats. var. *intermedia* Wats. Proc. Am. Acad. 23: 251. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 117. 1895.

Cespitose perennial, densely stellate throughout, stellae small, rays distinct or irregularly coherent, forked at the base; caudex much branched; stems stout, erect or decumbent, 2–18 cm. long, unbranched; terminal bud developing a fertile stem or remaining undeveloped; radical leaves linear to linear-oblanceolate, thick, usually involute, entire, 1–7 cm. long; cauline leaves similar, rather remote; petals yellow, narrowly spatulate, about 1 cm. long; filaments linear; fruiting inflorescence crowded and subcorymbiform; pedicels straight or slightly curved, 1–1.5 cm. long, horizontal or ascending; pods sessile, ovate, stellate, 4–6 mm. long, acute but not compressed at the apex; styles 2.5–6 mm. long; septum entire, nerved, areolae slightly tortuous; ovules 3–8 in each cell, funiculi attached to septum for about one-half their lengths; seeds not winged.

Distribution: southeastern Colorado, northern New Mexico, southern Utah, Arizona.

## Specimens examined:

Colorado: 25 miles below Manitou, May 28, 1878, *Jones 114* (Rky. Mt. Herb., U. S. Nat. Herb., and Mo. Bot. Gard. Herb.); Canyon City, April, 1871, *Brandeggee 370* (Mo. Bot. Gard. Herb.); Canyon City, June 26, 1895, *Osterhout 786* (Rky. Mt. Herb. and Geo. Osterhout Herb.).

New Mexico: Santa Fe, 1847, *Fendler 23, 38* (Mo. Bot. Gard. Herb.); hills at Santa Fe, May 13, 1897, *Heller 3516* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Utah: Rabbit Valley, July 25, 1875, *Ward 418* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Jugtown, June 5, 1894, *Jones 5404* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); S. Utah, 1872, *Bishop* (U. S. Nat. Herb.); Marysvale, May 31, 1894, *Jones 5338e* (U. S. Nat. Herb.); Marysvale, June 1, 1894, *Jones 5355a* (U. S. Nat. Herb.); Marysvale, June 4, 1894, *Jones 5388b* (U. S. Nat. Herb.); road to Panguitch Lake, Sept. 5, 1894, *Jones 5996c* (U. S. Nat. Herb.); canyon above Tropic, May 28, 1894, *Jones 5312d, 5312e* (U. S. Nat. Herb.).

Arizona: Eldon Mountain, July 11, 1891, *MacDougal* (U. S. Nat. Herb.); Clear Creek Canyon, May 9, 1901, *Ward* (U. S. Nat. Herb.); Moran Point, Grand Canyon, June 9, 1901, *Ward* (U. S. Nat. Herb.); Grand Canyon, May 24, 1903, *Grant 938* (Rky. Mt. Herb.); Grand Canyon, June 29, 1913, *Hitchcock 24* (U. S. Nat. Herb.); rim of Grand Canyon, July 1, 1915, *Hitchcock* (U. S. Nat. Herb.); Grand Canyon, June, 1915, *Macbride & Payson 952* (Rky. Mt. Herb.); common, San Francisco Mountains, May 10, 1908, *Tidestrom 964* (U. S. Nat. Herb.); vicinity of Flagstaff, July 2, 1898, *MacDougal 203* (U. S. Nat. Herb. and Rky. Mt. Herb.); Slate Mountains, near Flagstaff, June, 1900, *Purpus 7096* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); near Flagstaff, May-Oct., 1902, *Purpus* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); near Flagstaff, June 23, 1901, *Leiberg 5553* (U. S. Nat. Herb.); vicinity of Flagstaff, May 26-27, 1908, *Rose 12111* (U. S. Nat. Herb.); Walnut Canyon, vicinity of Flagstaff, Aug. 7-11, 1915, *Hitchcock* (U. S. Nat. Herb.); common on rocky slopes, 10 miles east of Jerome Junction, May 1, 1908, *Tidestrom 909* (U. S. Nat. Herb.); Fort Apache, April 15, *Shuttleworth* (U. S. Nat. Herb.).

This plant is undoubtedly distinct from *L. alpina*. Its coarser

habit of growth, more numerous ovules, straight or slightly curved pedicels, and more completely inflated pods separate it from both *alpina* and *condensata*. It is not so easily distinguished from *L. arizonica*, however, and further knowledge of these two species may result in the reduction of *intermedia* to varietal rank under *arizonica*. Such a change would seem unfortunate because *intermedia* is a plant of wide distribution, while *arizonica* is quite limited in range. It is believed also that the latter species has been derived from *intermedia*—a relationship that would not be suggested by making the parent group a variety of the derived form.

39. *L. arizonica* Wats. Proc. Am. Acad. 23: 254. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 117. 1895; Armstrong, Field Book of Western Wild Flowers, 184. 1915.

Cespitose perennial, densely stellate throughout, stellae small, rays distinct or irregularly coherent, forked at the base; caudex much branched; stems usually erect, 1.5–8 cm. long, unbranched; terminal bud either developing a fertile stem or remaining undeveloped; radical leaves linear to oblanceolate, flat, entire, .5–2.5 cm. long, the lowermost usually noticeably shorter than the ones immediately above; cauline leaves linear to oblanceolate, 1–2.5 cm. long; petals yellow, narrowly spatulate, 6–7 mm. long; filaments linear; fruiting inflorescence crowded and sub-corymbiform; pedicels straight or slightly curved, 4–8 mm. long; pods sessile, erect or ascending, stellate, ovate, 4–6 mm. long, acute but scarcely compressed at the apex; styles 1–2 mm. long; septum entire, nerved, arcolae slightly tortuous; ovules 4 in each cell, funiculi attached to septum for one-half their lengths or less; seeds not winged.

Distribution: northwestern Arizona.

Specimens examined:

Arizona: Mokiak Pass, 1877, *Palmer 43* (Mo. Bot. Gard. Herb.); Juniper Mountain, central Arizona, April, 1876, *Palmer 16* (Mo. Bot. Gard. Herb.); Ash Fork, May, 1883, *Rusby 514½* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Hackberry, May 26, 1884, *Jones 4371* (U. S. Nat. Herb.).

*L. arizonica* is very closely related to *L. intermedia* and is distinguished from it by the flat, shorter basal leaves and the



shorter style. It is apparently a more slender plant than the preceding species.

**39a. Var. nudicaulis Payson.<sup>1</sup>**

Perennial, silvery stellate throughout; caudex much branched, stems erect, 1-4 cm. long, naked; terminal bud apparently remaining undeveloped; radical leaves linear to linear-oblongate, mostly less than 1 cm. long; petals yellow; fruiting inflorescence subcorymbiform; pods sessile, stellate, about 4 mm. long, sometimes compressed at the apex; styles 1-2 mm. long; ovules 4-5 in each cell.

Distribution: northern Arizona.

Specimens examined:

Arizona: Buckskin Mountains, June 19, 1890, *Jones* (U. S. Nat. Herb. TYPE).

Fig. 25. *L. arizonica* var. *nudicaulis*. Habit sketch  $\times \frac{1}{2}$ . Trichomes  $\times 25$ .

A most interesting plant resembling superficially some of the perennial, scapose *Drabas*, but undoubtedly closely allied to *L. arizonica*. The leafless stems are, of course, its distinguishing feature.

**40. *L. alpina* (Nutt.) Wats.** Proc. Am. Acad. 23: 251. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 117. 1895; Rydb. Mem. N. Y. Bot. Gard. 1: 179. 1900; Rydb. Fl. Colo. 155. 1906; Nelson in Coulter & Nelson, Manual Cent. Rocky Mountains, 219. 1909; Nelson, Spring Fl. Intermountain States, 65. 1912; Clements & Clements, Rocky Mountain Flowers, 25. 1914; Rydb. Fl. Rocky Mountains, 332. 1917.

*Vesicaria alpina* Nutt. in Torr. & Gray, Fl. N. Am. 1: 102. 1838; Walp. Rep. 1: 141. 1842; Dietr. Gen. Pl. 3: 638. 1843; Hooker's London Jour. Bot. 6: 70. 1847; Coulter, Manual Rocky Mountain Region, 25. 1885.

*Alyssum alpinum* Kuntze, Rev. Gen. Pl. 2: 931. 1891.

*Lesquerella parvula* Greene, Pittonia 4: 308. 1901; Rydb. Fl. Colo. 155. 1906; Rydb. Fl. Rocky Mountains, 332. 1917.

Cespitose perennial, stellate throughout, stellae small, rays

<sup>1</sup> *Lesquerella arizonica* Wats. var. *nudicaulis*, var. nov., perennis humilis; caulibus 1-4 cm. longis, nudatis; siliculis ovatis, stellato-pubescentibus, sessilibus.—Collected in the Buckskin Mountains, Arizona, June 19, 1890, *M. E. Jones* (U. S. Nat. Herb.).

numerous, irregularly coherent, branching; caudex much branched; stems erect, 2-14 cm. long, unbranched; terminal bud apparently never developing into a fertile stem; radical leaves linear to linear-oblongate, entire, 1-4 cm. long; cauline leaves similar; petals yellow, narrowly spatulate, 5-7 mm. long; filaments linear, slightly enlarged at the base; fruiting inflorescence usually elongated; pedicels sigmoid, 5-10 mm. long; pods erect or ascending, sessile, ovate, compressed at the apex, 4-5 mm. long, stellate; styles 2-4 mm. long; septum frequently perforate, nerved, areolae tortuous or nearly straight; ovules 2-4 in each cell, funiculi attached to septum for one-half their lengths or more; seeds not winged.

Distribution: in the mountains of Montana, Wyoming, and northern Colorado.

Specimens examined:

Montana: without definite locality, *Kelsey 92* (Mo. Bot. Gard. Herb.); mountains about Helena, June 6, 1887, *Anderson* (Mo. Bot. Gard. Herb.); dry uplands, Helena, May 19, 1905, *Blankinship 58* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Bridger Canyon, June 16, 1901, *Jones* (Rky. Mt. Herb.); Greycliff, Sweet Grass County, May 25-31, 1912, *Eggleston 7835* (U. S. Nat. Herb.); Wreck Creek, Greycliff, Sweet Grass County, May 29, 1912, *Eggleston 7853* (U. S. Nat. Herb.); Trail Creek, Park County, July 2, 1899, *Blankinship* (Mo. Bot. Gard. Herb.); north slope of Baldy Mountain, Absaroka Range, Park County, June 25, 1912, *Eggleston 8072* (U. S. Nat. Herb.); Spanish Basin, Gallatin County, June 23, 1897, *Rydberg & Bessey 4170* (Geo. Osterhout Herb., U. S. Nat. Herb., and Rky. Mt. Herb.); west Gallatin River, June 9, 1899, *Jones* (U. S. Nat. Herb.); Sedan, Gallatin County, May 14, 1901, *Jones* (U. S. Nat. Herb.).

Wyoming: Teton River, June 14, 1854, *Doty 166* (Mo. Bot. Gard. Herb.); forks of Green River, July 6, 1860, *Hayden* (Mo. Bot. Gard. Herb.); Hillon's, near Colorado line, July 5-10, 1896, *Osterhout 1104* (Geo. Osterhout Herb.).

Colorado: lat. 39-41°, July, 1864, *Parry* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); summit of Mt. Bross, Middle Park, July 29, 1876, *Patterson* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Sulphur Springs, Grand County, June 9, 1906, *Osterhout 3260* (Geo. Osterhout Herb.); Troublesome, Grand County,

July 13, 1905, *Osterhout 3029* (Geo. Osterhout Herb. and Rky. Mt. Herb.).

**40a. Var. *spathulata* (Rydb.) Payson, new comb.**

*Lesquerella spathulata* Rydb. Contr. U. S. Nat. Herb. 3: 486. 1896; Britton & Brown, Ill. Fl. 2: 136. 1897, ed. 2, 2: 154. 1913; Rydb. Mem. N. Y. Bot. Gard. 1: 179. 1900; Petersen, Fl. Nebraska, 62. 1912; Rydb. Fl. Rocky Mountains, 332. 1917; Bergman, Fl. North Dakota, 191. 1918.

*Vesicaria alpina* Macoun, Cat. Canadian Pl. 1: 54. 1883.

*Lesquerella alpina* Webber, Cat. Fl. Nebraska, 119. 1890.

*L. nodosa* Greene, Pittonia 4: 309. 1901.

Stems 3–12 cm. long; radical leaves oblanceolate to ovate, 1–4 cm. long; petals yellow; filaments linear; pedicels sigmoid, 5–10 mm. long; pods ovate, acute, usually compressed at the apex; ovules 2–4 in each cell.

Distribution: Northwest Territory, Saskatchewan, Alberta, Montana, western North and South Dakota, northwestern Nebraska, and northeastern Wyoming.

Specimens examined:

Northwest Territory: Cypress Hills, Aug. 4, 1880, *Macoun* (U. S. Nat. Herb.).

Saskatchewan: Wood Mountain Post, Assiniboia, June 17, 1895, *Macoun 10511* (Mo. Bot. Gard. Herb.); Milk River, Assiniboia, July 13, 1895, *Macoun 10313* (Mo. Bot. Gard. Herb.).

South Dakota: Short Pines, Harding County, June 9, 1911, *Visher 444* (Rky. Mt. Herb.); summit of Eagle Nest Butte, Washabaugh County, May 30, 1914, *Over 2008* (U. S. Nat. Herb.); Bad Lands, July, 1855, *Hayden* (Mo. Bot. Gard. Herb.).

Montana: hills, Midvale, June 17, 1903, *Umbach 85* (U. S. Nat. Herb.); Duck Lake, June 23, 1901, *Weller* (U. S. Nat. Herb.); Falls of the Missouri, May, 1879, *Havard* (U. S. Nat. Herb.); Great Falls, May 31–July 13, 1888, *Williams 6* (U. S. Nat. Herb.); high, sterile chalk hills on the Yellowstone, 1853–4, *Hayden* (Mo. Bot. Gard. Herb.); Deer Lodge Valley, July 19, 1905, *Jones* (U. S. Nat. Herb.); dry upland benches, Anaconda, May 19, 1906, *Blankinship 659* (U. S. Nat. Herb.); Custer, May 4, 1890, *Blankinship 30* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Wyoming: railroad right-of-way, Moorcroft, Aug. 2, 1901, *Nelson 8553* (Rky. Mt. Herb.); Gillette, June 9, 1897, *Rydb. & Bessey 4169* (U. S. Nat. Herb.); rolling plains between Sheridan and Buffalo, June 15–July 15, 1900, *Tweedy 3587* (Rky. Mt. Herb.).

Nebraska: Belmont, July 18, 1889, *Webber* (Mo. Bot. Gard. Herb.).

Numerous intermediates between typical *spathulata* and *alpina* make specific separation of the two seem impossible. The only consistent difference between them lies in the leaf outline, a character scarcely deserving more than varietal recognition were there fewer intermediates or a greater separation of ranges. *L. alpina* and its variety are separated from *L. arizonica* and *L. intermedia* by the distinctly sigmoid rather than nearly straight pedicels, by the fewer ovules, and by the more slender habit of growth. The elongated fruiting inflorescence is the most striking difference between these forms and *L. condensata* with its variety *laevis*.

41. *L. condensata* A. Nelson, Bull. Torr. Bot. Club 26: 238. 1899; Nelson in Coulter & Nelson, Manual Cent. Rocky Mountains, 219. 1909; Nelson, Spring Fl. Intermountain States, 64. 1912; Rydb. Fl. Rocky Mountains, 332. 1917.

Densely caespitose perennial, pubescence rather loosely stellate, rays rather long, branched, distinct, not closely appressed to the plant surface; caudex much branched; stems erect, 1–3 cm. long, unbranched; terminal bud apparently always remaining undeveloped; radical leaves linear to linear-oblongate, entire, .5–2.5 cm.

long; cauline leaves few, similar; petals yellow, linear-spatulate, 5–7 mm. long; filaments linear, slightly broader at the base; fruiting inflorescence short, rarely exceeding the leaves; pedicels usually sigmoid, 3–7 mm. long; septum perforate or entire,



Fig. 26. *L. condensata*. Habit sketch  $\times \frac{2}{3}$ . Trichomes  $\times 25$ .

nerved, areolae usually more or less tortuous; ovules 2 in each cell, funiculi short, attached to the septum for about one-half their lengths; seeds not winged.

Distribution: southwestern Wyoming.

Specimens examined:

Wyoming: Tipton, June 17, 1898, *Nelson 4797* (Rky. Mt. Herb., TYPE); Green River, June 1, 1897, *Nelson 3071* (Rky. Mt. Herb.); Green River, June 25, 1895, *Shear 4369* (U. S. Nat. Herb.); stony hills, Kemmerer, June 1, 1907, *Nelson 9002* (Rky. Mt. Herb. and Mo. Bot. Gard. Herb.); Kemmerer, June 13, 1898, *Nelson 4675* (Mo. Bot. Gard. Herb.); head of Wind River Valley, May 31, 1860, *Hayden* (Mo. Bot. Gard. Herb.); stony slopes in the foothills, Laramie, May 30, 1900, *Nelson 6954* (Mo. Bot. Gard. Herb., Baker Herb. at Pomona College, Rky. Mt. Herb., and U. S. Nat. Herb., in part).

#### 41a. Var. *laevis* Payson.<sup>1</sup>

Cespitose perennial, silvery stellate throughout, rays short, branched, irregularly coherent, appressed; caudex much branched; terminal bud apparently remaining undeveloped; pedicels 2-5 mm. long, straight or slightly curved; pods often compressed at the apex; ovules 2 in each cell; septum entire or perforate, areolae nearly straight.

Distribution: eastern Wyoming and southern Montana.

Specimens examined:

Montana: on gravelly, clay slopes, 10 miles east of Monida, Madison County, June 18, 1899, *A. Nelson & E. Nelson 5428* (U. S. Nat. Herb., Rky. Mt. Herb., and Mo. Bot. Gard. Herb.); Cottonwood Creek, July 30, 1896, *Flodman 497* (U. S. Nat. Herb.); ridge above Bannock City, July 19, 1880, *Watson 32* (U. S. Nat. Herb.).

Wyoming: Platte Canyon, June 27, 1901, *Goodding* (Rky. Mt. Herb.); Laramie Hills, May 21, 1892, *Buffum 66* (Rky. Mt. Herb.); Laramie Hills, May 4, 1894, *Nelson 62* (U. S. Nat. Herb. and Rky. Mt. Herb.); Laramie Hills, May 18, 1895, *Nelson 1218* (Mo. Bot. Gard. Herb., TYPE, Rky. Mt. Herb., and U. S. Nat. Herb.); Laramie Hills, May 30, 1898, *Nelson 4324* (U. S. Nat. Herb.); stony slopes in the foothills, Laramie,

<sup>1</sup> *Lesquerella condensata* (Nutt.) Wats. var. *laevis*, var. nov., folia siliculaeque adpresse pubescentia; caulibus quam foliis brevioribus.—Collected in the Laramie Hills, Albany County, Wyoming, May 18, 1895, *A. Nelson 1218* (Mo. Bot. Gard. Herb.).

May 30, 1900, *Nelson 6954* (Mo. Bot. Gard. Herb., Rky. Mt. Herb., U. S. Nat. Herb. in part, and Geo. Osterhout Herb.); Freezeout Hills, July 11, 1898, *E. Nelson 4854* (Rky. Mt. Herb.).

With the exception of one collection (*Nelson 6954*), typical *condensata* is recorded only from western Wyoming. Since no other collections of *condensata* have been made at Laramie and since it is quite possible that the collections from the western and eastern parts of the state were inadvertently mixed in the distribution, it is assumed that *condensata* is confined to western Wyoming, and that the variety *laevis* occurs in eastern Wyoming and southern Montana, but not within the range of typical *condensata*. Due to an unfortunate error in distribution the type collection of the variety *laevis* was labelled *Draba glacialis* and consequently may be located under that name in herbaria.

*L. condensata* and its variety differ from *alpina* chiefly in the much-reduced stems and in the constant reduction of their ovules to two in each cell. The variety *laevis* is distinguished from the typical form by the character of the pubescence, and although this at first may seem of slight consequence, it appears to be invariable and is so characteristic that the two may be easily separated without the aid of a lens. These forms are two of a number of interesting pulvinate plants, not uncommon on the high plains of southern Wyoming, that give to the vegetation an aspect quite alpine, an appearance that is always a little surprising at so low an altitude.

#### 42. *L. Garrettii* Payson.<sup>1</sup>

Perennial, stellate-pubescent throughout, stellae small, rays numerous, forked near the base, distinct or irregularly coherent; stems very slender, erect or decumbent, unbranched, 3-5 cm. long; terminal bud apparently developing a fertile stem; radical leaves 1-3 cm. long, blade entire, obtuse, spatulate or narrowly elliptical, gradually narrowed to the very slender and much longer petiole; flowers 3-7; petals very narrowly spatulate,

<sup>1</sup>*Lesquerella Garrettii* sp. nov., perennis humilis pube lepto-stellata undique argentea; caulibus tenuissimis erectis vel procumbentibus simplicibus 3-5 cm. longis; foliis radicalibus integris spatulatis vel sublineari-oblongis 1-3 cm. longis; pedicellis erectis vel leviter sinuosis; siliculis subglobosis, circiter 4 mm. diametro stellato-pubescentibus, distincte stipitatis; stylis 4-5 mm. longis; loculis 4-ovulatis; funiculis septo adnatis; seminibus immarginatis.—Type collected in Big Cottonwood Canyon, Salt Lake County, Utah, June 28, 1905, by A. O. Garrett 1344 (Mo. Bot. Gard. Herb.).

yellow, 6-7 mm. long; filaments linear, slightly larger at the base; fruiting inflorescence short; pedicels usually with a tendency to become sigmoid, 3-6 mm. long; pods erect or ascending, densely stellate-pubescent, rays of stellae not appressed, subsessile, or distinctly stipitate, subglobose, 3-4 mm. long; stipe black, less than 1 mm. long, styles slender, 4-5 mm. long; septum nerved, perforate, areolae not tortuous; ovules 4 in each cell, funiculi attached to the septum for one-half their lengths or less; seeds not margined.

Distribution: Salt Lake County, Utah.

Specimens examined:

Utah: in clefts in rock on mountain side, Big Cottonwood Canyon, Salt Lake County, June 28, 1905, *Garrett 1344* (Mo. Bot. Gard. Herb., TYPE, and Rky. Mt. Herb.).

This plant is apparently most nearly related to *L. alpina* (Nutt.) Wats., but is at once separated from that species by the presence of a definite stipe. The pods are subglobose and inflated in the new species, while in *alpina* they are conspicuously elongated and compressed at the apex. In general appearance also it is unlike *alpina* because of its much more slender, lax stems. The name is given in honor of the collector, Prof. A. O. Garrett, of Salt Lake City, Utah, who writes me that he has collected this plant at an altitude of 9700 feet in rich, wet, loamy soil in a granitic locality from which the snow had but recently melted.

43. *L. cinerea* Wats. in Gray, Syn. Fl. N. Am. 1': 118. 1895.

*L. ? cinerea* Wats. Proc. Am. Acad. 23: 255. 1888.

Perennial, densely stellate-pubescent throughout, stellae conspicuously granular, rays many, distinct, forked near the base; stems prostrate or decumbent, unbranched, 6-12 cm. long; terminal bud remaining undeveloped; radical leaves 1-2.5 cm. long, spatulate or oblanceolate, entire or obscurely repand, obtuse or acute; cauline leaves numerous, oblanceolate, 5-15 mm. long; petals yellow, frequently turning reddish on fading, narrowly spatulate, 8-10 mm. long; filaments linear, slightly enlarged at the base; fruiting inflorescence elongated, occasionally somewhat leafy; pedicels straight or somewhat sigmoid, 5-9 mm. long; pods erect or ascending, sessile, densely stellate-

pubescent, ellipsoid, inflated, slightly obcompressed, particularly when young, about 5 mm. long; styles shorter than the pods; septum nerved, areolae not tortuous; ovules 7-12 in each cell, funiculi attached to septum for less than one-half their lengths; seeds not winged.

Distribution: Arizona.

Specimens examined:

Arizona: without definite locality, 1869, *Palmer* (U. S. Nat. Herb.); Belmont, June 29, 1892, *Toumey* 65 (U. S. Nat. Herb.); dry soil near Kendrick Mountains, June 28, 1901, *Leiberg* 5599 (U. S. Nat. Herb.); common on slopes near Elgin, April 11, 1908, *Tidestrom* 823 (U. S. Nat. Herb., in part).

This interesting plant, though possessing few characteristic peculiarities, is evidently quite distinct from other known species of the genus. It is to be associated with *Kingii* and its relatives, and like them develops a dense rosette. It may be distinguished from any of them by the spatulate basal leaves in which the blade tapers gradually to the broad petiole. No specimens have been seen in completely mature condition. It is apparently a very rare plant.

44. *L. Kingii* Wats. Proc. Am. Acad. 23: 251. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>st</sup>: 117. 1895.

*Vesicaria Kingii* Wats. Proc. Am. Acad. 20: 353. 1885.

Perennial, silvery stellate throughout, stellae small, rays few to numerous, distinct or irregularly coherent, forked at the base; stems rather lax, 1-2 dm. long, usually unbranched; terminal bud remaining undeveloped; radical leaves 1.5-7 cm. long, blade ovate to suborbicular, entire, obtuse, narrowed abruptly to the slender petiole which usually exceeds the blade in length; cauline leaves oblanceolate, .5-2 cm. long, entire, obtuse or acute; petals narrowly spatulate, yellow or fading purplish, 7-8 mm. long; filaments linear; fruiting inflorescence elongated; pedicels horizontal or recurved, decidedly sigmoid, 8-12 mm. long;



Fig. 27. *L. cinerea*. Habit sketch  $\times \frac{1}{3}$ . Trichomes  $\times 25$ .



Pods erect or horizontal, subsessile or shortly stipitate, subglobose, not compressed, 3–5 mm. in diameter, rather sparingly stellate-pubescent; styles slender, equaling or slightly exceeding the pods; septum nerved, areolae not tortuous; ovules 2–4, funiculi short, attached to the septum for less than one-half their lengths; seeds not winged.



Fig. 28. *L. Kingii*. Habit sketch  $\times \frac{1}{2}$ . Trichomes  $\times 25$ .

Distribution: Nevada and southeastern California.

Specimens examined:

Nevada: west Humboldt Mountains, June, 1868, *Watson 82* (Gray Herb., TYPE, photograph Mo. Bot. Gard. Herb., and tracing Rky. Mt. Herb.); Big Creek and Kingston Canyon, Toiyabe Forest, July 28, 1913, *Hitchcock 807* (U. S. Nat. Herb.); dry ground, Bunker Hill, Toiyabe Forest, July 29, 1913, *Hitchcock 848, 868* (U. S. Nat. Herb.); rocky slopes, Mt. Sabb, Palmetto Range, May–Oct., 1898, *Purpus 5863* (U. S. Nat. Herb. and Baker Herb. at Pomona College).

California: Telescope Peak, Panamint Mountains, June 23, 1891, *Coville & Funston 2025* (Gray Herb. and U. S. Nat. Herb.).

*L. Kingii* was the first of a number of forms to be described from the region of the Great Basin that are here being treated as species. These are *L. Wardii*, *L. prostrata*, *L. utahensis*, and *L. latifolia*. The differences between them are slight and within their specific limits they show considerable variation. There is evidently here a remarkable plexus of evolution due perhaps to some germinal plasticity or perhaps to the topographical character of the country that isolates races on every detached mountain range. However the presence of such an assemblage of minute forms may be explained, the result remains difficult of treatment by the taxonomist. Perhaps they were best regarded as varieties under one great species, but in the present case this might easily result in a polyphyletic group and so emphasize an unnatural relationship.

*L. Kingii*, because of its subglobose pods, will be confused with *utahensis* rather than the other members of this group. It is a less distinctly caespitose plant than *utahensis*, has as a rule fewer stems, and is less floriferous, the leaves are apparently always entire, and the petals exceed the sepals by not more than one-third their lengths. The pedicels are more distinctly sigmoid, the pods more densely pubescent than in *utahensis* and are never compressed at right angles to the septum. *Kingii* has not yet been collected in Utah, while *utahensis* has never been found outside that state. The habit sketch reproduced here was drawn from the type of *Kingii*, while the fragment of fruiting inflorescence is taken from Hitchcock's No. 807.

45. *L. latifolia* A. Nelson, Bot. Gaz. 42: 49. 1906.

Perennial, appressed stellate-pubescent throughout, rays of the stellae numerous, distinct or irregularly coherent, usually forked near the base; stems erect or decumbent, 1-2 dm. long, unbranched, rather stout; terminal bud remaining undeveloped; radical leaves 2-8 cm. long, obtuse, blade suborbicular, ovate or oblong, entire or irregularly margined, narrowed abruptly to a slender petiole 1.5-5 cm. long; cauline leaves broadly oblanceolate to spatulate, obtuse, 1-2 cm. long; flowers numerous, conspicuous; petals yellow, narrowly spatulate, 7-9 mm. long; filaments linear; fruiting inflorescence elongated; pedicels conspicuously sigmoid, 5-7 mm. long, horizontal or even recurved; pods erect, stellate-pubescent, oblong, 5-7 mm. long, somewhat flattened parallel to the partition, distinctly stipitate, stipe black, about 5 mm. long; styles 2-3 mm. long; septum nerved, entire, areolae not tortuous; ovules 6 in each cell, funiculi long and slender, attached to the septum for less than one-half their lengths; seeds not margined.

Distribution: southern Nevada.

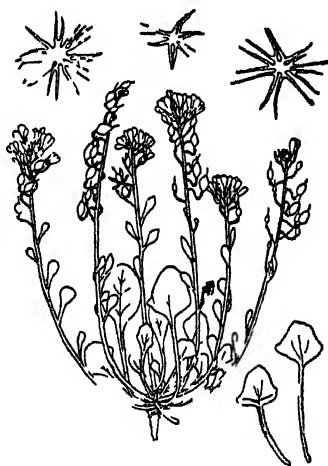


Fig. 29. *L. latifolia*. Habit sketch  $\times \frac{1}{3}$ . Trichomes  $\times 25$ .

## Specimens examined:

Nevada: mountain tops, Karshaw, Meadow Valley Wash, April 26, 1902, *Goodding 625* (Rky. Mt. Herb., TYPE, and Mo. Bot. Gard. Herb.).

This is certainly a most unusual plant and further collections will be awaited with interest. In general appearance it is not unlike *L. Kingii* except for the more floriferous racemes and more numerous stems. It is definitely separated from that species, however, by the lengthened pods and the more distinct stipe. The pods in the type specimen are scarcely mature but seem evidently flattened parallel to the septum. The type collection was distributed under the name of *L. montana*.

46. *L. Wardii* Wats. in Gray, Syn. Fl. N. Am. 1: 118. 1895; Rydb. Fl. Rocky Mountains, 332. 1917.

*L. ? Wardii* Wats. Proc. Am. Acad. 23: 255. 1888.

Perennial, densely stellate-pubescent throughout, stellae small, many-rayed, rays forked near the base, distinct or irregularly co-

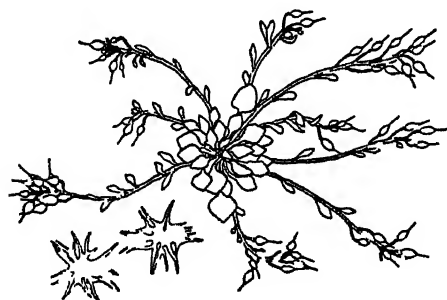


Fig. 30. *L. Wardii*. Habit sketch  $\times \frac{1}{15}$ .  
Trichomes  $\times 25$ .

herent; stems mostly prostrate, 4–10 cm. long, stiff, unbranched; terminal bud remaining undeveloped; radical leaves 1–4 cm. long, blade ovate, suborbicular or rarely subhastate, usually quite entire, narrowed abruptly to the slender petiole which equals or exceeds it in length; cauline leaves broadly oblanceolate to

nearly linear, 8–18 mm. long; petals yellow, very narrowly spatulate, about 7 mm. long; filaments linear, slightly enlarged at the point of attachment; fruiting inflorescence rather short, crowded; pedicels straight and erect or ascending or, particularly the lower ones, horizontal and more or less sigmoid, 5–7 mm. long; pods erect or ascending, sessile or subsessile, ovoid or ellipsoid, usually acute at the apex, sometimes slightly flattened at right angles to the septum, rather densely stellate-pubescent, 4–10 mm. long; styles 3–5 mm. long, usually

shorter than the mature pod; septum nerved, entire or perforate, areolae polygonal or somewhat tortuous; ovules 2-8 in each cell, funiculi attached to the septum for about one-half their lengths; seeds not margined, radical turned slightly to one side.

Distribution: in the mountains of south central Utah and western Nevada.

Specimens examined:

Utah: Mt. Ellen, Henry Mountains, July 24, 1894, *Jones 5667c* (U. S. Nat. Herb.); Mt. Ellen Peak, Henry Mountains, July 25, 1894, *Jones 5684e* (U. S. Nat. Herb.); Bromide Pass, Mt. Ellen, Henry Mountains, July 27, 1894, *Jones 5695b* (U. S. Nat. Herb.); Aquarius Plateau, Aug. 16, 1875, *Ward 589* (Mo. Bot. Gard. Herb.).

Nevada: spring in desert near Goshoot Mountains, May 8, 1859, *H. Engelmann 90* (Mo. Bot. Gard. Herb.); ridge south side of Lee Canyon, Charleston Mountains, in limestone, Clark County, July 26, 1913, *Heller 11004* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Rush Valley, May 2, 1859, *H. Engelmann 94* (Mo. Bot. Gard. Herb.).

*L. Wardii* forms a dense and strikingly symmetrical rosette and is evidently a plant of higher, more exposed localities than is *utahensis*. It also differs from that species, besides in the characters elsewhere mentioned, in having the shorter, solitary stamens incompletely surrounded at the base by the nectar glands. In fruit characters it is quite similar to *L. prostrata*, but unlike that species, has entire, obtuse leaves.

47. *L. utahensis* Rydb. Bull. Torr. Bot. Club 30: 252. 1903; Rydb. Fl. Rocky Mountains, 333. 1917.

Perennial, stellate-pubescent throughout, stellae small, rays numerous, forked at or near the base, distinct or irregularly coherent; stems decumbent or procumbent, 5-20 cm. long, unbranched; terminal bud remaining undeveloped; radical leaves 2-5 cm. long, blade ovate to oblong, entire, subhastate or rarely fiddle-shaped, usually obtuse, narrowed abruptly to the slender petiole which usually exceeds the blade in length; cauline leaves broadly oblanceolate to spatulate, entire, 5-15 mm. long; flowers numerous, showy; petals yellow or sometimes tinged with red, narrowly spatulate, 7-9 mm. long; filaments

slightly broadened at the base; fruiting inflorescence elongated;



Fig. 31. *L. utahensis* Habit sketch  $\times \frac{1}{3}$ .  
Trichomes  $\times 25$ .

pedicels ascending, horizontal or even recurved, usually with a tendency to become sigmoid, 4-10 mm. long; pods erect to horizontal, subsessile, 3-5 mm. in diameter, sparsely stellate-pubescent, sometimes nearly glabrous, more or less ob-compressed, apex usually truncate and rarely slightly emarginate, in some speci-

mens referred here pods subglobose; styles very slender, 4-5 mm. long; septum entire or perforate, nerved, areolae not tortuous; ovules 2-6 in each cell, funiculi attached to septum for less than one-half their lengths; seeds not margined.

Distribution: in the mountains of Utah.

Specimens examined:

Utah: Logan Peak, Cache County, July 4, 1910, *Smith 2248*, 2244 (Rky. Mt. Herb.); Brigham Peak, Aug. 29, 1894, *Jones 5958u* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); rocky ridges, Dyer Mine, Uintah Mountains, July 5, 1902, *Goodding 1258* (Rky. Mt. Herb., U. S. Nat. Herb., and Mo. Bot. Gard. Herb.); Big Cottonwood Canyon, between Silver Lake and the summit of Mt. Majestic, June 28, 1905, *Rydberg & Carlton 6411* (Rky. Mt. Herb. and U. S. Nat. Herb.); in clefts in exposed rocks, Big Cottonwood Canyon, Salt Lake County, July 1, 1905, *Garrett 1370* (U. S. Nat. Herb.); American Fork Canyon, July 31, 1880, *Jones 1354* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); common on rocky ridges along Ephraim sheep trail, July 1, 1908, *Tidestrom 1322* (U. S. Nat. Herb.); plateau east of Ephraim Canyon, Aug. 14, 1907, *Tidestrom 203* (U. S. Nat. Herb.); head of Salina Canyon, June 15, 1894, *Jones 5441* (Mo. Bot. Gard. Herb. and Rky. Mt. Herb.); Marysvale, June 2, 1894, *Jones 5375e* (U. S. Nat. Herb.); Marysvale in Bullion Canyon, June 5, 1894, *Jones 5397b* (Mo. Bot. Gard. Herb., Rky. Mt. Herb., and U. S. Nat. Herb.); mountains north of Bullion Creek, near Marysvale, July 23, 1905, *Rydberg & Carl-*

ton 7160 (Rky. Mt. Herb. and U. S. Nat. Herb.); Panguitch Lake, Sept. 6, 1894, *Jones 6002e* (U. S. Nat. Herb.); canyon above Tropic, May 28, 1894, *Jones 5312d* (U. S. Nat. Herb.).

*L. utahensis* is perhaps the most interesting of all the species of *Lesquerella* because of the great similarity, in some of its forms particularly, to members of the genus *Physaria*. So striking, indeed, is this similarity that one is a little perplexed at times to know to which genus a given plant should be referred. And yet *utahensis* as a species is not entirely satisfactory, so close is it to other forms that give no suggestion of *Physaria*. The bridge connecting the two genera is nearly complete.

*L. utahensis*, as here limited, is rather polymorphic and when more adequate collections are at hand it may be capable of resolution into several geographic varieties. This species seems most closely related to *Wardii* but that species has, when mature, large, irregularly ellipsoid pods. Both are conspicuous rosette formers. *L. Kingii* is perhaps more difficult to separate from *utahensis* than is *Wardii*. It has a more southern and western range, the stems are longer and more nearly erect, and the pods more densely pubescent. *L. prostrata* differs from the present species in the somewhat elongated pods and the acute, frequently subhastate leaves.

48. *L. prostrata* A. Nelson, Bull. Torr. Bot. Club 26: 124. 1899; Nelson in Coulter & Nelson, Manual Cent. Rocky Mountains, 219. 1909; Rydb. Fl. Rocky Mountains, 332. 1917.

Perennial, silvery stellate-pubescent throughout, stellae many-rayed, rays forked near the base, distinct; stems prostrate or ascending, unbranched, 6–15 cm. long; terminal bud re-

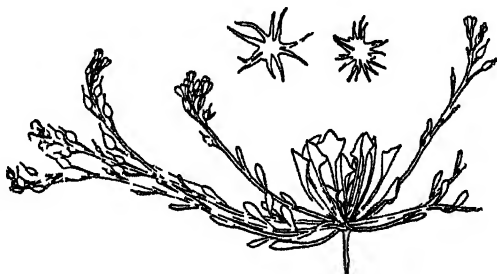


Fig. 32. *L. prostrata*. Habit sketch  $\times \frac{1}{3}$ . Trichomes  $\times 25$ .

maining undeveloped; radical leaves ovate or subhastate, obtuse or acute, usually distinctly angular, 1–3.5 cm. long, blade abruptly narrowed to the slender petiole which usually exceeds it in length;

cauline leaves oblanceolate to linear, 8–15 mm. long; petals yellow, narrowly spatulate, 6–7 mm. long; filaments slightly and gradually broadened toward the base; fruiting inflorescence elongated; pedicels erect or ascending, straight or slightly sigmoid, 5–8 mm. long; pods erect or ascending, sessile, rather sparsely stellate-pubescent, ovoid, not compressed, usually acute at the apex, 5–6 mm. long; styles rather stout, 3–4 mm. long; septum nerved, perforate, areolae not tortuous; ovules 2–3 in each cell, funiculi attached to the septum for less than one-half their lengths; seeds not winged.

Distribution: southwestern Wyoming and southern Idaho.

Specimens examined:

Wyoming: Piedmont, June 7, 1898, *Nelson 4564* (Rky. Mt. Herb., TYPE, and Mo. Bot. Gard. Herb.).

Idaho: open stony slopes near base of peak, south end of Soldier Mountains, June 26, 1916, *Macbride & Payson 2897* (Mo. Bot. Gard. Herb., Rky. Mt. Herb., and U. S. Nat. Herb.).

*L. prostrata* and *L. utahensis* are certainly very closely related, and it is quite possible that collections showing characters intermediate between the two may be made in northern Utah. In the Idaho specimen the septum is so largely perforate that only a narrow margin remains around the replum.

49. *L. diversifolia* Greene, *Pittonia* 4: 309. 1901.

Perennial, densely stellate-pubescent throughout with many-rayed stellae, rays distinct, forked near the base; caudex enlarged, clothed with the persistent leaf-bases of previous years, frequently branched; stems usually prostrate, 4–15 cm. long, unbranched; terminal bud remaining undeveloped; radical leaves 2–6 cm. long, blade entire, ovate to nearly orbicular, usually



Fig. 33. *L. diversifolia*. Habit sketch  $\times \frac{1}{8}$ . Trichomes  $\times 25$ .

rather abruptly narrowed to the petiole, obtuse or acutish; cauline leaves narrowly oblanceolate, rather few, 5–25 mm. long; petals

yellow, narrowly spatulate, 7 mm. long; filaments linear; fruiting inflorescence elongated; pedicels conspicuously sigmoid, 5–10 mm. long; pods horizontal to erect, sessile, densely stellate-pubescent, circular, oblong or obovate, flattened somewhat parallel to the septum, compressed at the apex and along the margins, 4–6 mm. long; septum nerved, entire or perforate, areolae more or less tortuous; ovules 2 in each cell, funiculi attached to the septum for less than one-half their lengths; seeds not margined or winged, radical turned slightly to one side.

Distribution: in the mountains of central Idaho and eastern Oregon.

Specimens examined:

Idaho: Lost River Mountains, Aug. 14, 1895, *Henderson 3885* (U. S. Nat. Herb.); divide of Warm Spring and Little Smoky Creeks, Sawtooth Mountains, Aug. 7, 1909, *Woods & Tidestrom 2728* (U. S. Nat. Herb.); Sawtooth National Forest, 1910, *Woods 56a* (Rky. Mt. Herb.); loose, sliding slopes, Smoky Mountains, Aug. 13, 1916, *Macbride & Payson 3770* (Rky. Mt. Herb., U. S. Nat. Herb., and Mo. Bot. Gard. Herb.).

Oregon: dry mountain sides, Wallowa Mountains, Aug. 5, 1899, *Cusick 2304* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); granitic soil, extreme source of Imnaha River, Wallowa Mountains, Aug., 1906, *Cusick 3135* (U. S. Nat. Herb., Rky. Mt. Herb., and Mo. Bot. Gard. Herb.); alpine sliding sands, Wallowa Mountains, *Cusick 3700* (U. S. Nat. Herb.); Steins Mountains, near Wild Horse Creek, July 15, 1898, *Cusick 2036* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

This essentially alpine plant is characterized by the enlarged caudices, dense rosettes, and short stems. In fruit characters it is very similar to *L. occidentalis* and might with propriety be considered varietally under that species. The two plants are rather easily separated, and since their ranges also seem not to merge it was thought advisable to retain the original treatment.

50. *L. occidentalis* Wats. Proc. Am. Acad. 23: 251. 1888; Wats. in Gray, Syn. Fl. N. Am. 1<sup>1</sup>: 117. 1895; Howell, Fl. Northwest Am. 51. 1897; Piper, Contr. U. S. Nat. Herb. 11: 298. 1906; Piper & Beattie, Fl. Northwest Coast, 176. 1915.



*Vesicaria montana* Wats. Bot. Calif. 1: 43. 1876, and suppl. 2: 432. 1880.

*V. occidentalis* Wats. Proc. Am. Acad. 20: 353. 1885.

*Physaria montana* Greene, Fl. Franciscana, 249. 1891.

Perennial, silvery stellate-pubescent throughout with many-rayed stellae, rays forked near the base; caudex more or less enlarged, woody; stems decumbent to erect, 1-2 dm. long, unbranched; terminal bud remaining undeveloped; radical leaves 2-7 cm. long, blade ovate or narrower, tapering gradually to the petiole, entire, frequently repand or even sublyrate; cauline leaves oblanceolate, entire, 1-1.5 cm. long; petals yellow, narrowly spatulate, 9-10 mm. long; filaments linear; fruiting inflorescence elongated; pedicels conspicuously sigmoid, 8-12 mm. long; pods usually erect, sessile, densely stellate-pubescent, oblong to obovate, flattened somewhat parallel to the partition, compressed at the apex and along the margins, 4-6 mm. long; styles 4-5 mm. long; septum entire or perforate, nerved, areolae somewhat tortuous; ovules usually 2 in each cell, funiculi attached for about one-half their lengths; seeds not margined, radical somewhat turned to one side.

Distribution: northeastern California and adjacent Oregon.

Specimens examined:

Oregon: Steins and southern Blue Mountains, July 21, 1898, *Cusick 2054* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Mitchell, May 14, 1885, *Howell* (U. S. Nat. Herb.).

California: Humbug Hills near Yreka, June 30, 1876, *Greene 902* (Gray Herb., TYPE, photograph Mo. Bot. Gard. Herb.); Marble Mountain, Siskiyou County, June, 1901, *Chandler 1653* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); Greenhorn Mountain, Siskiyou County, May 15, 1910, *Butler 1342* (U. S. Nat. Herb. and Rky. Mt. Herb.); loose rocky ground, mountain on Truckee River, Placer County, June, 1887, *Sonne 23* (U. S. Nat. Herb.).

The typical *L. occidentalis*, as distinguished from the segregates *L. diversifolia* and *L. Cusickii*, possesses stout stems that carry even the lowermost pods of the fruiting inflorescence well beyond the longest radical leaves. The caudex, although definitely perennial, is not so densely clothed with leaf bases as is *diversifolia*, due perhaps to a less distinctly alpine habitat.

**51. *L. Cusickii* Jones, Contr. Western Botany 12: 2. 1908.**

Annual or short-lived perennial, densely stellate-pubescent throughout, stellae many-rayed, rays forking near the base, distinct or irregularly coherent; stems numerous, unbranched, 4-20 cm. long; terminal bud remaining undeveloped; radical leaves 2-6 cm. long, blade suborbicular, ovate or broadly oblanceolate, entire or repand, frequently abruptly narrowed to the slender petiole; cauline leaves 1-2.5 cm. long, entire or repand, oblanceolate; petals yellow, narrowly spatulate, 7-8 mm. long; filaments linear; fruiting inflorescence elongated; pedicels horizontal or even recurved, conspicuously sigmoid, 6-14 mm. long; pods sessile, erect to horizontal, densely stellate-pubescent, suborbicular to obovate, somewhat flattened parallel to the septum, compressed at the apex and on the margins, 4-6 mm. long; styles 2-4 mm. long; septum entire, nerved, areolae somewhat tortuous; ovules 2 in each cell, funiculi attached for about one-half their lengths; seeds not margined.

Distribution: Oregon.

Specimens examined:

Oregon: Fossil, Gilliam County, May 30, 1894, *Leiberg* 130 (U. S. Nat. Herb., Rky. Mt. Herb., and Mo. Bot. Gard. Herb.); white clay hills of Willow Creek, Malheur County, May 3, 1900, *Cusick* 2367 (U. S. Nat. Herb., Rky. Mt. Herb., and Mo. Bot. Gard. Herb.); banks of Otis Creek, Malheur County, June 20, 1896, *Leiberg* 2337 (U. S. Nat. Herb.).

*L. Cusickii*, although agreeing with *occidentalis* in the characters of the pods, is definitely separated from it and *diversifolia* by the short-lived root. It apparently occurs only on white clay soils at a low altitude and to most of the herbarium specimens the white soil still adheres.

**52. *L. Douglasii* Wats. Proc. Am. Acad. 23: 255. 1888; Howell, Fl. Northwest Am. 52. 1897; Piper, Contr. U. S. Nat. Herb. 11: 298. 1906; Henry, Fl. Southern British Columbia, 145. 1915.**

*Vesicaria ludoviciana* Hook. Fl. Bor. Am. 1: 48. 1840; Torr. Wilkes' U. S. Expl. Exp. 17: 232. 1874.

Perennial, silvery stellate throughout, stellae many-rayed, rays forked at the base, distinct or irregularly coherent; caudex

usually unbranched; stems numerous, erect or decumbent, un-

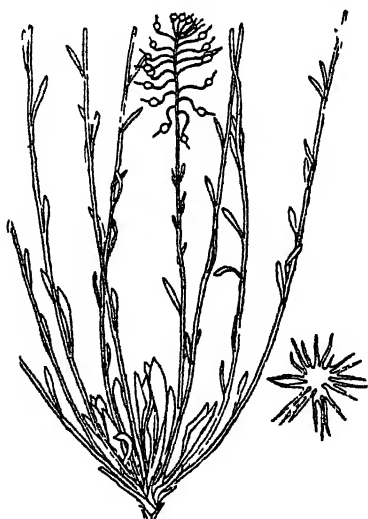


Fig. 34. *L. Douglasii*. Habits sketch  $\times \frac{1}{8}$ . Trichomes  $\times 25$ .

branched, 1–4.5 dm. long; terminal bud remaining undeveloped; radical leaves 3–10 cm. long, blade obovate to very narrowly oblanceolate, entire, repand or with a few conspicuous teeth, acute or obtuse, tapering gradually to a long slender petiole; cauline leaves narrowly oblanceolate to linear, 1–5 cm. long, entire or very shallowly toothed; petals narrowly spatulate, 6–9 mm. long; filaments linear; fruiting inflorescence elongated; pedicels 6–15 mm. long, usually horizontal, straight or sigmoid, the lowermost frequently recurved; pods erect, horizontal or rarely pendent, sessile,

rather sparsely stellate-pubescent, globose or slightly elongated, not compressed, 3–4 mm. in diameter; styles slender, equalling or longer than the pods; septum nerved, areolae somewhat tortuous; ovules 2–4 in each cell, funiculi attached to the septum for about one-half their lengths; seeds not margined.

Distribution: southern British Columbia, central Washington, and northern Oregon.

Specimens examined:

British Columbia: Lake Osoyoos, June 7, 1905, *Macoun 70853* (Mo. Bot. Gard. Herb.).

Washington: Conconully, eastern Washington, June, 1902, *Griffiths & Cotton 312* (U. S. Nat. Herb.); without definite locality, 1889, *Vasey 186* (U. S. Nat. Herb.); upper Columbia, *Wilkes 857* (U. S. Nat. Herb.); gravelly hillside north of Wenatchee River, May 14, 1899, *Whited 1065* (U. S. Nat. Herb.); rocky bar of Columbia at Wenatchee, June 2, 1899, *Whited 1119* (U. S. Nat. Herb.); near Wenatchee, May 24, 1900, *Whited 1247* (U. S. Nat. Herb.); rocky bar of Columbia River, Wenatchee, May 14, 1905, *Whited 2606* (U. S. Nat. Herb.); Rock Island, Kittitas County, July 10, 1893, *Sandberg & Leiberg 426* (U. S.

Nat. Herb.); banks of the Columbia River near Columbus, April 14, June, 1886, *Suksdorf 842* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.).

Oregon: Columbia River near Umatilla, May 1, 1882, *Howell* (U. S. Nat. Herb. and Mo. Bot. Gard. Herb.); along Columbia River at Heppner Junction, April 16, 1903, *Lunell* (U. S. Nat. Herb.); Biggs, Sherman County, May 31, 1910, *Heller 10114* (U. S. Nat. Herb.).

*L. Douglasii* is a definitely limited species marking the farthest migration of the genus to the northwest. Its closest relative apparently is *L. occidentalis*. From this species and its relatives it is at once separated by the inflated pods that are not at all compressed at the margins and by the taller, more nearly erect stems. The geographical distribution, so far as available specimens show, is peculiarly limited to the Valley of the Columbia River.

#### SPECIES EXCLUDED

*Lesquerella velebitica* Degen, Magyar Bot. Lap. 8: 3. 1909 = *Degenia velebitica* (Degen) Hayek, Oesterr. Bot. Zeitschr. 60: 93. 1910.

This interesting plant from the Balkans is of strikingly similar aspect to certain species of *Lesquerella* but is certainly not to be regarded as having been derived from the same group of *Cruciferae* as they. Its elevation to generic rank seems a satisfactory disposition of it.

*Lesquerella thlaspiiformis* (Phil.) Gilg & Muschler in Engl. Bot. Jahrb. 42: 466. 1909 = *Eudema thlaspiiforme* Phil. Anal. Univ. Chile, 675. 1872.

This plant is unknown to the author but from the description seems not to be referable to *Lesquerella*. It is a native of the province of Santiago, Chile.

*Lesquerella flexuosa* Brandege, Zoe 5: 233. 1906.

The relationship of this plant will be treated in a subsequent paper. Its affinity is certainly not with *Lesquerella*.

#### LIST OF EXSICCATAE

In the following index to the specimens cited in this monograph the collector's number, if one occurs, is printed in italics

and is followed immediately by a number in parentheses. The latter number indicates the serial number of the species involved as adopted in the present study. The name of this species follows the parentheses.

Anderson, F. W.

(40) *L. alpina*; (21) *L. argentea*.

Baker, C. F.

2 (15) *L. Fendleri*; 354 (32) *L. rectipes*; (33) *L. montana*.

Baker, C. F., Earle, F. S. & Tracy, S. M.

85 (32) *L. rectipes*; 901 (33) *L. montana*.

Baker, C. F. & Holzinger, J. M.

92 (33) *L. montana*.

Baker, H. P.

(21) *L. argentea*.

Ball, J.

1697 (5) *L. auriculata*; (21) *L. argentea*; (33) *L. montana*.

Bandelier, A. F.

(15) *L. Fendleri*.

Barnes, S. O.

(1) *L. Lescurii*.

Bates, J. M.

(21) *L. argentea*.

Beals, I. M.

(27) *L. Gordonii*.

Beard, A.

(27) *L. Gordonii*.

Bergman, H. F.

1875 (21) *L. argentea*.

Berlandier, J. L.

819, 884, 2239, 2314 (13) *L. Berlandieri*; 2538 (6) *L. grandiflora*; 3102, 3017

(2a) *L. lasiocarpa* var. *Berlandieri*.

Bethel, E.

(9) *L. ovalifolia*; (30) *L. pruinosa*.

Bigelow, J. M.

(15) *L. Fendleri*.

Biltmore Herbarium.

1292, 2695a (33) *L. montana*; 2693a (26) *L. gracilis*; 4273, 4273a (35) *L. globosa*; 6966a (18) *L. recurvata*; 14807 (6) *L. grandiflora*.

Bishop, Capt.

(38) *L. intermedia*.

Blankinship, L. A.

(5) *L. auriculata*.

Blankinship, J. W.

30, 659 (40a) *L. alpina* var. *spathulata*; 58 (40) *L. alpina*; (40) *L. alpina*; (24) *L. angustifolia*.

Bodin, J. E.

63 (18) *L. recurvata*.

Bogue, E. E.

(26) *L. gracilis*.

Brandegee, T. S.

25 (33) *L. montana*; 345 (15) *L. Fendleri*; (38) *L. intermedia*.

Bray, W. L.

107, 285 (7) *L. densiflora*; 304 (12) *L. argyrea*.

Broadhead, G. C.

105 (33) *L. montana*.

- Buffum, B. C.  
     60 (33) *L. montana*; 61 (21) *L. argentea*; 66 (41a) *L. condensata* var. *laevis*.  
 Burk, W. H.  
     7 (11) *L. arctica*.  
 Bush, B. F.  
     186 (26) *L. gracilis*; 1152 (26a) *L. gracilis* var. *repanda*; 1170 (18) *L. recurvata*.  
 Butler, G. D.  
     1342 (50) *L. occidentalis*.  
 Canby, W. M.  
     18 (15) *L. Fendleri*; 21 (12) *L. argyrea*; 25 (18) *L. recurvata*.  
 Carleton, M. A.  
     214 (27) *L. Gordonii*.  
 Carr, W. P.  
     8 (22) *L. arenosa*.  
 Chandler, H. P.  
     1153 (50) *L. occidentalis*.  
 Churchill, J. R.  
     (33) *L. montana*.  
 Clemens, Mr. and Mrs. J.  
     807 (18) *L. recurvata*.  
 Clements, F. E.  
     2693 (21) *L. argentea*.  
 Clifton, R. L.  
     3023 (8) *L. Engelmannii*.  
 Coues, E. & Palmer, E.  
     183, 197, 237 (27) *L. Gordonii*.  
 Coville, F. V. & Funston, P.  
     406 (28) *L. Palmeri*; 2025 (44) *L. Kingii*.  
 Cowles, H. C.  
     62 (22) *L. arenosa*.  
 Crandall, C. S.  
     212 (33) *L. montana*.  
 Cusick, W.  
     2036, 2304, 3135, 3700 (49) *L. diversifolia*; 2054 (50) *L. occidentalis*; 2367 (51) *L. Cusickii*.  
 Dodds, G. S.  
     1889 (33) *L. montana*.  
 Dodge, C. K.  
     61 (2) *L. lasiocarpa*.  
 Doty, J.  
     166 (40) *L. montana*.  
 Drejer, S. T. N.  
     (11) *L. arctica*.  
 Eastwood, A.  
     8 (15) *L. Fendleri*.  
 Eggert, H.  
     (12) *L. argyrea*; (7) *L. densiflora*; (8) *L. Engelmannii*; (15) *L. Fendleri*; (27) *L. Gordonii*; (26) *L. gracilis*; (2) *L. lasiocarpa*; (9) *L. ovalifolia*; (18) *L. recurvata*.  
 Eggleston, W. W.  
     4419 (1) *L. Lescurii*; 5632, 11195, 17552 (33) *L. montana*; 7335, 7853, 8072 (40) *L. alpina*; 9030 (21) *L. argentea*.  
 Ellis, C. C.  
     7 (29) *L. pinetorum*.  
 Emig, W. H.  
     498 (9) *L. ovalifolia*.  
 Engelmann, G.  
     781 (26a) *L. gracilis* var. *repanda*; (21) *L. argentea*.  
 Engelmann, H.  
     90, 94 (46) *L. Wardii*.

Fendler, A.

38, 33 (38) *L. intermedia*; 39, 40 (15) *L. Fendleri*.

Fernald, M. L. &amp; St. John, H.

216 (11a) *L. arctica* var. *Purshii*.

Fernald, M. L. &amp; Wiegand, K. M.

3465 (11a) *L. arctica* var. *Purshii*.

Fiebig, K.

3034, 2619 (36) *L. mendocina*.

Fisher, C. L.

104 (9) *L. ovalifolia*; 105 (15) *L. Fendleri*.

Flodman, J. H.

497 (41a) *L. condensata* var. *laevis*.

Garrett, A. O.

1344 (42) *L. Garrettii*; 1370 (47) *L. utahensis*.

Gattinger, A.

(35) *L. globosa*; (1) *L. Lescurii*.

Gleason, H. A.

(21) *L. argentea*.

Goodding, L. N.

26, 56 (21) *L. argentea*; 61 (14) *L. purpurea*; 74, 2228 (15) *L. Fendleri*; 625 (45) *L. latifolia*; 1258 (47) *L. utahensis*; 2155, 2184 (28) *L. Palmeri*; (41a) *L. condensata* var. *laevis*.

Gordon, A.

(27) *L. Gordonii*.

Grant, G. B.

983 (38) *L. intermedia*.

Greene, E. L.

902 (50) *L. occidentalis*; (27) *L. Gordonii*; (33) *L. montana*; (9) *L. ovalifolia*.

Greenman, J. M., Jr. &amp; Greenman, M. T.

91 (27) *L. Gordonii*.

Gregg, J.

90, 292, 315 (12) *L. argyrea*; 91, 304 (15) *L. Fendleri*.

Griffiths, D.

3493, 3531, 3548, 3905, 4011, 4091 (27) *L. Gordonii*; 3646, 4146 (14) *L. purpurea*; 4014, 4250 (15) *L. Fendleri*.

Griffiths, D. &amp; Cotton, J. S.

312 (52) *L. Douglasii*.

Hall, E.

19 (7) *L. densiflora*; 20 (18) *L. recurvata*; 21 (8) *L. Engelmannii*; 22 (26) *L. gracilis*; 23 (6) *L. grandiflora*.

Hall, E. &amp; Harbour, J. P.

48 (21) *L. argentea*; 49 (33) *L. montana*.

Hall, H. M.

5845, 5882 (28) *L. Palmeri*.

Harris, J. A.

C142 (27) *L. Gordonii*; C1485 (14) *L. purpurea*.

Hartman, C. V.

615 (15) *L. Fendleri*.

Havard, V.

72 (15) *L. Fendleri*; (40a) *L. alpina* var. *spathulata*; (12) *L. argyrea*; (15) *L. Fendleri*; (2) *L. lasiocarpa*; (14) *L. purpurea*.

Hayden, F. V.

88 (21) *L. argentea*; (40) *L. alpina*; (40a) *L. alpina* var. *spathulata*; (21) *L. argentea*; (41) *L. condensata*.

Hayes, I. I.

10 (11) *L. arctica*.

Heller, A. A.

1405 (2) *L. lasiocarpa*, in part; 1405 (2a) *L. lasiocarpa* var. *Berlandieri*, in part; 1478 (25) *L. Lindheimeri*; 1657 (18) *L. recurvata*; 10114 (46) *L. Wardii*.

- Heller, A. A. & Heller, E. G.  
 3509 (33) *L. montana*; 3516 (38) *L. intermedia*; 3576 (15) *L. Fendleri*; 3634 (32) *L. rectipes*; (33) *L. montana*.
- Henderson, L. F.  
 3885 (49) *L. diversifolia*.
- Herrick, C. L.  
 204, 531 (29) *L. pinetorum*; 304 (14) *L. purpurea*; 537 (15) *L. Fendleri*.
- Hitchcock, A. S.  
 16 (21) *L. argentea*; 24 (38) *L. intermedia*; 807, 848, 858 (44) *L. Kingii*; 1077 (9) *L. ovalifolia*; (38) *L. intermedia*; (9) *L. ovalifolia*.
- Hough, W.  
 59 (15) *L. Fendleri*.
- Houghton, H. W.  
 3573 (9) *L. ovalifolia*.
- Howell, A. H.  
 332 (12) *L. argyraea*; 356 (6) *L. grandiflora*.
- Howell, T. J.  
 (52) *L. Douglasii*; (50) *L. occidentalis*.
- Hubbard, G. W.  
 185 (1) *L. Lescurii*.
- Jermey, G.  
 (12) *L. argyraea*; (15) *L. Fendleri*; (26b) *L. gracilis* var. *sessilis*; (18) *L. recurvata*.
- Johnson, J. E.  
 (28) *L. Palmeri*.
- Johnston, E. L.  
 164 (21) *L. argentea*; 850 (33) *L. montana*; 976 (33a) *L. montana* var. *suffruticosa*.
- Jones, B. J.  
 (40) *L. alpina*.
- Jones, M. E.  
 19 (33) *L. montana*; 114, 5312e, 5338e, 5355a, 5388b, 5404, 5996c (38) *L. intermedia*; 1354, 5312d, 5375e, 5397b, 5441, 5958u, 6002e (47) *L. utahensis*; 3722 (14) *L. purpurea*; 3879, 5024e, 5029b (28) *L. Palmeri*; 4371 (39) *L. arizonica*; 5297a (21) *L. argentea*; 5302a (15) *L. Fendleri*; 5667c, 5684e, 5695b (46) *L. Wardii* (40a) *L. alpina* var. *spathulata*; (39a) *L. arizonica* var. *nudicaulis*; (21) *L. argentea*.
- Jones, W. W.  
 (40) *L. alpina*.
- Joor, J. F.  
 93, 95 (26) *L. gracilis*.
- Jørgensen, P.  
 1062 (36) *L. mendocina*.
- Kelsey, F. D.  
 92 (40) *L. alpina*.
- Kennedy, P. B.  
 1096 (28) *L. Palmeri*.
- Kenoyer, L. A.  
 (26a) *L. gracilis* var. *repanda*.
- Knowlton, F. H.  
 94, 134 (21) *L. argentea*.
- Leiberg, J. B.  
 130, 2337 (51) *L. Cusickii*; 5553 (38) *L. intermedia*; 5599 (43) *L. cinerea*.
- Lemmon, J. G.  
 (14) *L. purpurea*.
- Lesquereux, L.  
 (35) *L. globosa*; (1) *L. Lescurii*.
- Letterman, G. W.  
 (27) *L. Gordonii*.
- Lewton, F. L.  
 118 (26) *L. gracilis*.
- Lindheimer, F.  
 8, 12, 330 (18) *L. recurvata*; 217 (5) *L. auriculata*; 299, 331, 668 (26) *L. gracilis*;



- 326, 301, 669 (26b) *L. gracilis* var. *sessilis*; 303, 329, 367, 666, 667, 670 (12) *L. argyraea*; 325, 421, 526, 576 (8) *L. Engelmannii*; 327 (25) *L. Lindheimeri*; 328, 577 (7) *L. densiflora*; (5) *L. auriculata*; (6) *L. grandiflora*.
- Lloyd, F. E.  
326 (15) *L. Fendleri*.
- Lundarr, A.  
(12) *L. arctica*.
- Lunnell, J.  
(22) *L. arenosa*; (52) *L. Douglasii*.
- Macbride, J. F. & Payson, E. B.  
952 (38) *L. intermedia*; 2397 (48) *L. prostrata*; 3770 (49) *L. diversifolia*.
- MacDougal, D. T.  
203 (38) *L. intermedia*; (38) *L. intermedia*.
- Macoun, J. M.  
10313, 10511 (40a) *L. alpina* var. *spathulata*; 12401 (22) *L. arenosa*; 70853 (52) *L. Douglasii*.
- Marsh, C. C.  
81 (32) *L. rectipes*.
- Marsh, C. D.  
(21) *L. argentea*.
- Maxon, W. R.  
3815 (6) *L. grandiflora*.
- Mearns, E. A.  
3 (15) *L. Fendleri*; 4 (14) *L. purpurea*; 5 (27) *L. Gordonii*; 1246, 1336 (12) *L. argyraea*.
- Mell, C. D.  
(21) *L. argentea*.
- Mell, C. D. & Knopf  
(21) *L. argentea*.
- Merrill, E. D. & Wilcox, E. N.  
521, 733 (21) *L. argentea*; 568 (23) *L. macrocarpa*.
- Metcalf, J. K.  
48 (27) *L. Gordonii*.
- Metcalf, O. B.  
23 (27) *L. Gordonii*; 1534 (29) *L. pinetorum*.
- Mohr, P. F.  
(27) *L. Gordonii*.
- Moodie, M. E.  
810 (22) *L. arenosa*.
- Mulford, A. I.  
614 (15) *L. Fendleri*.
- Nealley, G. C.  
121a, 268, 477 (14) *L. purpurea*; 147 (2) *L. lasiocarpa*; 148 (26) *L. gracilis*; 700, 701 (15) *L. Fendleri*.
- Nelson, A.  
59, 190, 1310, 3949, 7275, 8284 (21) *L. argentea*; 62, 1218, 4324, 6954, in part (41a) *L. condensata* var. *laevis*; 88, 1370, 3757, 7256, 8842 (33) *L. montana*; 2424 (34) *L. curvipes*; 3071 (41) *L. condensata*; 4564 (48) *L. prostrata*; 4675, 4797, 6954, 9002 (41) *L. condensata*, in part; 7081 (23) *L. macrocarpa*; 8553 (40a) *L. alpina* var. *spathulata*; (33) *L. montana*.
- Nelson, A. & Nelson, E.  
5428 (41a) *L. condensata* var. *laevis*; (34) *L. curvipes*.
- Nelson, E.  
265 (33) *L. montana*; 4854 (41a) *L. condensata* var. *laevis*.
- Nelson, E. W.  
3919 (15) *L. Fendleri*; 6631 (13) *L. Berlandieri*; 6771 (12) *L. argyraea*.
- Newton, Dr.  
(26a) *L. gracilis* var. *repanda*.
- Orcutt, C. R.  
1099 (28) *L. Palmeri*; 6106 (15) *L. Fendleri*; (12) *L. argyraea*.
- Osterhout, G. E.  
785, 3851, 4426, 4998, 5615, 5889 (33) *L. montana*; 786 (38) *L. intermedia*; 1102, 1103, 2621, 2864, 5888, 5914 (21) *L. argentea*; 1104, 3029, 3260 (40) *L.*

- alpina; 1947 (32) *L. rectipes*; 2050, 3965, 4412 (15) *L. Fendleri*; 4878 (9) *L. ovalifolia*; 5781 (33a) *L. montana* var. *suffruticosa*.
- Over, W. H.  
2005 (21) *L. argentea*; 2008 (40a) *L. alpina* var. *spathulata*; 6268 (22) *L. arenosa*.
- Oyster, J. H.  
(26a) *L. gracilis* var. *repanda*.
- Pace, L.  
44 (26) *L. gracilis*; 55 (18) *L. recurvata*; 210 (7) *L. densiflora*.
- Palmer, E.  
9, 86 (14) *L. purpurea*; 10 (27) *L. Gordonii*; 16 (39) *L. arizonica*; 29 (16) *L. Schaffneri*; 30, 182, 372, 464 (12) *L. argyreae*; 31, 182½, 277, 558 (15) *L. Fendleri*; 33, 41, 366 (2) *L. lasiocarpa*; 43 (39) *L. arizonica*; 570 (28) *L. Palmeri*; (21) *L. argentea*; (43) *L. cinerea*.
- Palmer, E. J.  
9121, 11219 (12) *L. argyreae*; 9136 (6) *L. grandiflora*; 9153, 10076, 11429 (7) *L. densiflora*; 9951 (18) *L. recurvata*; 11215, 11259 (2) *L. lasiocarpa*; 11479 (15) *L. Fendleri*; 11583, 11861 (18) *L. recurvata*; 11622 (26b) *L. gracilis* var. *sessilis*; 12526 (9) *L. ovalifolia*.
- Parish, W. F.  
(14) *L. purpurea*.
- Parry, C. C.  
(40) *L. alpina*.
- Parry, C. C., Bigelow, J. M., Wright, C. & Schott, A.  
42 (15) *L. Fendleri*; 43 (12) *L. argyreae*; 44 (27) *L. Gordonii*.
- Parry, C. C. & Palmer, E.  
25 (12) *L. argyreae*; 26 & 25½ (16) *L. Schaffneri*.
- Patterson, H. N.  
(40) *L. alpina*.
- Payson, E. B.  
294, 669 (32) *L. rectipes*; 1017 (33) *L. montana*; 1021 (15) *L. Fendleri*.
- Plank, E. N.  
(24) *L. angustifolia*.
- Plummer, F. G.  
(31) *L. lata*.
- Popenoe, E. A.  
(15) *L. Fendleri*.
- Porsild, M. P.  
(11) *L. arctica*.
- Porter, T. C.  
(21) *L. argentea*; (1) *L. Lescurii*.
- Pringle, C. G.  
176 (15) *L. Fendleri*; 949 (14) *L. purpurea*; 6399 (3) *L. Schaueriana*; 9132 (12) *L. argyreae*; 10236 (2) *L. lasiocarpa*; (27) *L. Gordonii*; (14) *L. purpurea*.
- Purpus, C. A.  
1024, 4920 (12) *L. argyreae*; 1025, 4926 (15) *L. Fendleri*; 1148, 5232 (16) *L. Schaffneri*; 3339 (17) *L. pueblensis*; 5863 (44) *L. Kingii*; 7096 (38) *L. intermedia*; (38) *L. intermedia*.
- Ramaley, F.  
710, 1027 (33) *L. montana*.
- Reverchon, J.  
40, 2970 (26) *L. gracilis*; 42 (26b) *L. gracilis* var. *sessilis*; 1439, 3718 (12) *L. argyreae*; 3967, 3717 (5) *L. auriculata*; 3716 (26a) *L. gracilis* var. *repanda*; 3719 (2) *L. lasiocarpa*; 4288 (27) *L. Gordonii*; (12) *L. argyreae*; (5) *L. auriculata*; (7) *L. densiflora*; (8) *L. Engelmannii*; (15) *L. Fendleri*; (27) *L. Gordonii*; (26) *L. gracilis*; (6b) *L. gracilis* var. *sessilis*; (6) *L. grandiflora*; (9) *L. ovalifolia*; (18) *L. recurvata*.
- Rose, J. N.  
42 (34) *L. curvipes*; 11652, 11692 (14) *L. purpurea*; 11740 (27) *L. Gordonii*; 12111 (38) *L. intermedia*.
- Rose, J. N. & Fitch, W. R.  
17908 (15) *L. Fendleri*.

- Rose, J. N. & Painter, J. H.  
6430 (15) *L. Fendleri*.
- Rose, J. N., Painter, J. H. & Rose, J. S.  
8350, 8315, 8901 (3) *L. Schaueriana*; 10027 (17) *L. pueblensis*.
- Rusby, H. H.  
14, 398 (27) *L. Gordonii*; 15 (14) *L. purpurea*; 16 (15) *L. Fendleri*; 5141½ (39) *L. arizonica*.
- Russell, C.  
(26) *L. gracilis*.
- Ruth, A.  
5 (9) *L. ovalifolia*; 39 (26) *L. gracilis*.
- Rutter, C.  
(21) *L. argentea*.
- Rydberg, P. A.  
22 (9) *L. ovalifolia*; 533 (22) *L. arenosa*; 1231 (21) *L. argentea*; (21) *L. argentea*.
- Rydberg, P. A. & Bessey, E. A.  
4169 (40a) *L. alpina* var. *spatulata*; 4170 (40) *L. alpina*.
- Rydberg, P. A. & Carlton, E. C.  
6411, 7160 (47) *L. utahensis*.
- Rydberg, P. A. & Garrett, A. O.  
8558, 9448 (32) *L. rectipes*.
- Rydberg, P. A. & Vreeland, F. K.  
6137, 6139, 6141, 6145 (33) *L. montana*; 6142 (9) *L. ovalifolia*; 5143 (15) *L. Fendleri*.
- Ryder, C.  
(11) *L. arctica*.
- Safford, W. E.  
1261 (15) *L. Fendleri*.
- Sandberg, J. H. & Leiber, J. B.  
426 (52) *L. Douglasii*.
- Sarvis, J. T.  
6 (21) *L. argentea*.
- Schaffner, J. G.  
555 (16) *L. Schaffneri*.
- Schneck.  
(33) *L. montana*.
- Sharp, S. S.  
339 (34) *L. curvipes*.
- Shear, C. L.  
4369 (41) *L. condensata*.
- Shuttleworth, E. A.  
(38) *L. intermedia*.
- Shepherd, T. M.  
(27) *L. Gordonii*; (9) *L. ovalifolia*.
- Skehan, J.  
3 (15) *L. Fendleri*; (27) *L. Gordonii*.
- Smith, C. P.  
2248, 2244 (47) *L. utahensis*.
- Sonne, C. F.  
23 (50) *L. occidentalis*.
- Standley, P. C.  
6054 (33) *L. montana*; 6249, 6294 (33a) *L. montana* var. *suffruticosa*; 7087 (32) *L. rectipes*; 7091 (15) *L. Fendleri*; (27) *L. Gordonii*; (14) *L. purpurea*.
- Stanton, E. M.  
(21) *L. argentea*.
- Stearns, E.  
137 (14) *L. purpurea*.
- Stein, R.  
170, 174 (11) *L. arctica*.
- Stevens, G. W.  
36, 348 (9) *L. ovalifolia*; 88 (26a) *L. gracilis* var. *repanda*; 188 (5) *L. auriculata*.

- Suksdorf, W. N.  
842 (52) *L. Douglasii*.
- Swift, Dr.  
(7) *L. densiflora*.
- Thorner, J. G.  
369 (27) *L. Gordonii*.
- Tidestrom, I.  
303, 1322 (47) *L. utahensis*; 823 (43) *L. cinerea*, in part; 827 (14) *L. purpurea*;  
909, 964 (38) *L. intermedia*; 2176 (21) *L. argentea*.
- Toumey, J. W.  
65 (43) *L. cinerea*; 66 (27) *L. Gordonii*; (27) *L. Gordonii*.
- Tracy, S. M.  
8043 (27) *L. Gordonii*; 8044 (15) *L. Fendleri*; 9193 (6) *L. grandiflora*; 9196  
(26) *L. gracilis*; 9348 (2a) *L. lasiocarpa* var. *Berlandieri*.
- Tracy, S. M. & Earle, F. S.  
31 (15) *L. Fendleri*; 119 (27) *L. Gordonii*; 143, 338 (15) *L. Fendleri*.
- Trelease, W.  
22 (12) *L. argyrea*; (12) *L. argyrea*; (26a) *L. gracilis* var. *repanda*; (18) *L. recurvata*.
- Tweedy, F.  
147 (33a) *L. montana* var. *suffruticosa*; 3537 (40a) *L. alpina* var. *spathulata*;  
3538 (34) *L. curvipes*; 4489 (21) *L. argentea*; 5067 (33) *L. montana*; (27) *L. Gordonii*.
- Umbach, L. M.  
85 (40a) *L. alpina* var. *spathulata*.
- Vasey, G. R.  
47 (21) *L. argentea*; 186 (52) *L. Douglasii*; (15) *L. Fendleri*; (27) *L. Gordonii*;  
(14) *L. purpurea*.
- Visher, S. S.  
176 (21) *L. argentea*; 444 (40a) *L. alpina* var. *spathulata*; 571 (22) *L. arenosa*.
- Waldron, C.  
123 (22) *L. arenosa*.
- Walker, E. P.  
150, 163 (32) *L. rectipes*.
- Ward, L. F.  
418 (38) *L. intermedia*; 539 (46) *L. Wardii*; (15) *L. Fendleri*; (38) *L. intermedia*.
- Watson, S.  
32 (41a) *L. condensata* var. *laevis*; 82 (44) *L. Kingii*.
- Webber, H. C.  
(40a) *L. alpina* var. *spathulata*; (21) *L. argentea*.
- Weller, S.  
(40a) *L. alpina* var. *spathulata*.
- White, M.  
140 (27) *L. Gordonii*.
- Whited, K.  
1065, 1119, 1247, 2606 (52) *L. Douglasii*.
- Williams, T. A.  
46, 333 (21) *L. argentea*; (21) *L. argentea*.
- Willits, V.  
94 (34) *L. curvipes*.
- Wilkes' Expedition.  
857 (52) *L. Douglasii*.
- Wilkinson, E. H.  
101 (18) *L. recurvata*; (14) *L. purpurea*.
- Wolfe, J.  
641 (33) *L. montana*; (21) *L. argentea*.
- Woods, C. N.  
56a (49) *L. diversifolia*.
- Woods, C. N. & Tidestrom, I.  
2723 (49) *L. diversifolia*.
- Wootton, E. O.  
155 (15) *L. Fendleri*; 245 (20) *L. aurea*; (20) *L. aurea*; (15) *L. Fendleri*; (7)

*L. Gordonii*; (31) *L. lata*; (28) *L. Palmeri*; (29) *L. pinetorum*; (14) *L. purpurea*; (32) *L. rectipes*.

Wootton, E. O. & Standley, P. C.

3460 (29) *L. pinetorum*; (15) *L. Fendleri*; (27) *L. Gordonii*.

Wright, C.

16, 849 (12) *L. argyrea*; 16, 850, 851, 852, 1319 (15) *L. Fendleri*; 848 (26) *L. gracilis*; 1318 (17) *L. Gordonii*; 1320 (14) *L. purpurea*; (5) *L. auriculata*.

York, H. H.

385 (26) *L. gracilis*.

Young, M. S.

28 (6) *L. grandiflora*; 96 (14) *L. purpurea*; (8) *L. Engelmannii*.

Zuck, M.

(15) *L. Fendleri*; (14) *L. purpurea*.

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## STUDIES IN THE PHYSIOLOGY OF THE FUNGI XIV. SULPHUR NUTRITION: THE USE OF THIOSULPHATE AS INFLUENCED BY HYDROGEN-ION CONCENTRATION<sup>1</sup>

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### INTRODUCTION AND HISTORICAL REVIEW

Sulphur is an essential element in the cell because it is a component part of certain indispensable proteins. The metabolism or decomposition of various sulphur compounds by bacteria has been investigated by several workers, but the metabolism of the fungi with reference to such compounds has been little studied and is poorly understood. The activity of the sulphofying organisms of the soil has been a field for considerable investigation in recent years. It has been shown that these organisms bring about the change of the organic sulphur of the soil into sulphates which become available for crops. The oxidation of sulphur by such organisms has been shown to be of value in making available the phosphorus of mineral phosphates (McLean, '18).

Sulphur metabolism may also play a beneficial role in sewage disposal in that certain species of bacteria may cause the oxidation of sulphur and of hydrogen sulphide to sulphates, thus reducing the amount of odor. Attempts have been made to apply

<sup>1</sup> An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfilment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

the production of hydrogen sulphide to water analysis, the assumption having been that the hydrogen sulphide produced is proportional to the pollution. The literature pertaining to such attempts will be found in the paper by Myers ('20). The fate of hydrogen sulphide produced in putrefactions and other processes under the influence of "sulphur bacteria" has also been investigated by several workers, chiefly Winogradsky ('87, '88), Keil ('12), Dügge ('19), and Skene ('14). In addition to the hydrogen sulphide produced in putrefactions, another source of this compound is from the reduction of sulphates by micro-organisms such as bacteria and yeasts. The literature pertaining to sulphate reduction by bacteria and yeasts is discussed in papers by Lederer ('13) and Tanner ('17, '18).

An interesting phenomenon resulting from the sulphur metabolism of many organisms is the deposition of sulphur in the cells. This has been shown for bacteria by Cramer ('70), Cohn ('75), Winogradsky ('88), Keil ('12), Miyoshi ('97), Hinze ('03), and Lidforss ('12). Wille ('02) has denied the occurrence of sulphur in *Thiothrix*. Jonsson ('89) was the first to describe sulphur in the hyphae of fungi. He describes refractive bodies which are not sulphur, but oily bodies containing sulphur, in the hyphae of *Penicillium glaucum* growing on N/10  $H_2SO_4$  solution. Raciborski ('06) and Kossowicz and Loew ('12) have also described the presence of sulphur in the hyphae of fungi.

That sulphocyanate compounds are available for the growth of some bacteria and fungi seems established from the work of Beijerinck ('04), Munro ('86), Czapek ('03), Puriewitsch ('12), Kossowicz and Gröller ('12), Fernbach ('02), and Sauton ('10), though Holschewnikoff ('89) did not observe a decomposition of such compounds by bacteria, and Nägeli ('82) states that ammonium sulphocyanate can not be assimilated by fungi.

The relation of many organisms to the thiosulphates has been investigated by a considerable number of workers. Hydrogen sulphide is most generally produced as a result of the action of the organisms on these compounds as was found by Holschewnikoff ('89), Beijerinck ('00, '04), Petri and Maassen ('93), Saltet ('00), Nathansohn ('02), Sasaki and Otsuka ('12), Lederer ('13), and Tanner ('17) in the case of bacteria; Neuberg and Welde ('15), Beijerinck ('95), Tanner ('18), and Stange ('15) in the case of yeasts; and Raciborski ('06) and Kossowicz and Loew ('12)

in the case of the fungi. Gehring ('15), Lockett ('14), and Lieske ('12), in their investigations with certain bacteria, report the use of the thiosulphate but not a production of hydrogen sulphide. Gehring found the gases produced to be 5.5 per cent of carbon dioxide and 94.5 per cent of nitrogen, while Lieske found 20 per cent carbon dioxide and 80 per cent nitrogen. Bokorny ('12) and Nägeli ('82), working respectively with yeasts and fungi, report the growth of the organisms on sodium thiosulphate. Buchner and Hahn ('03) have found that the juice from pressed yeast has a reducing action on sulphur and thiosulphate.

With filamentous fungi on the varying solutions containing thiosulphate employed by Raciborski and Kossowicz and Loew, other products than hydrogen sulphide as a result of the growth of the organisms were sulphates, sulphites, extracellular sulphur, intracellular sulphur, and polythionates. All of these substances, except the sulphite, for which no tests were made, were found in one or the other of my cultures. The crystallization of the sulphur in the old hyphae of *Aspergillus niger* in the form of double pyramids as reported by Raciborski, has also occurred noticeably in some of my cultures in which the concentration of the thiosulphate was 2 per cent or more.

It is only in very recent times that work has been done dealing with the actual hydrogen-ion concentration of media and the shifting of the hydrogen-ion concentration due to metabolism. Clark and Lubs ('17) have given the final reaction of a culture of *Aspergillus niger* as  $P_H$  1.7. Currie ('17) has given the critical hydrogen-ion concentration for *Aspergillus niger* on a solution very similar to that employed in experiments 16 to 21 of this paper as  $P_H$  1.4 to 1.6. Steinberg ('19) reported the final hydrogen-ion concentration for the same fungus on Pfeffer's solution as  $P_H$  1.0–2.0 in most cases. On a modified Pfeffer's solution, the highest hydrogen-ion concentration of any culture in my experiments was  $P_H$  1.5, though the final reaction was greatly influenced by the initial  $P_H$  of the medium. Meacham ('18) stated that the limiting acidity appears in the region of  $P_H$  1.7 for 4 wood-destroying fungi with which he worked. Zeller, Schmitz, and Duggar ('19) gave the changes in hydrogen-ion concentration of several liquid media due to the growth of a number of wood-destroying fungi. The general tendency was



thiosulphate content, growth, and the use of the sugar, a sufficient number of flasks were inoculated so that 3 flasks could be removed at stated intervals and the several determinations made from these cultures. It is impossible to make all the determinations throughout from the same flasks, which would be a preferable method if convenient, though the results obtained indicate that the method employed is satisfactory.

Hydrogen-ion concentrations were determined colorimetrically, using the standard solutions as recommended by Clark and Lubs ('17). As the color produced in the solution necessitated the use of a colorimeter, a DuBoscq micro-colorimeter was employed of the type and according to the method described by Duggar ('19).

### EXPERIMENTAL RESULTS

#### A. AVAILABILITY OF SOME COMPOUNDS AS SOURCES OF SULPHUR

The experimental work presented in this paper was in progress several years, experiments 1-15 having been completed during the period 1915-1917 at the University of Wisconsin and the remainder of the experiments at the Missouri Botanical Garden during the sessions 1919-20 and 1920-21. Several of the solutions of the first 15 experiments are the same as those used by Kossowicz and Loew and Kossowicz and Gröller. Only 15 of the 32 experiments performed during the first period of the work are presented, the results obtained being in more or less general agreement throughout. The cultures were placed in a large constant temperature dark room with a variation of from 22 to 25° C. The fungi have been designated in the tables by abbreviations as, *Aspergillus niger*, A. nig.; *Penicillium glaucum*, P. gl.; *Penicillium cyclopium*, P. cycl.; and *Botrytis cinerea*, B. cin.

The extent of the darkening of the lead acetate paper is taken as the criterion of H<sub>2</sub>S production which is expressed roughly by the figures 0, 1, 2, 3, 4. An attempt is made to give a general indication of the relative spore production by the use of the same figures. The tabulated data as to the presence of sulphates is given by the plus and minus signs without any attempt to express the relative production of such compounds. This system of notation appears in all the tables.

The nutrient solution used to obtain the results given in table

1 contained the following amounts of the salts per 50-cc. culture:  $\text{Na}_2\text{S}_2\text{O}_3$  M/10, 8.1 cc.;  $\text{KH}_2\text{PO}_4$  M/6, 1.1 cc.;  $\text{KNO}_3$  M/4, 3.9 cc.;  $\text{NH}_4\text{Cl}$  M/4, 3.7 cc.;  $\text{MgCl}_2$  M/60, 2.9 cc.;  $\text{FeCl}_3$  M/1000, .5 cc.; dextrose M/1, 2.8 cc. as the source of carbon in experiment 1; sucrose M/1, 2.8 cc. as the source of carbon in experiment 2. It will be observed from table 1 that where equi-molecular volumes of dextrose and sucrose are employed differences in

TABLE 1

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING SODIUM THIOSULPHATE. TIME INTERVAL OF CULTURES, 7 WEEKS

| Fungus                              | Experiment 1<br>.2 per cent $\text{Na}_2\text{S}_2\text{O}_3$ and dextrose |        |         |       | Experiment 2<br>.2 per cent $\text{Na}_2\text{S}_2\text{O}_3$ and sucrose |        |         |       |
|-------------------------------------|--|--------|---------|-------|---|--------|---------|-------|
|                                     | A. nig.  | P. gl. | B. cin. | Check | A. nig.   | P. gl. | B. cin. | Check |
| No. cultures                        | 2  | 2      | 2       | 1     | 3   | 4      | 2       | 1     |
| Dry wt. (gms.)                      | .0941  | .1351  | .1117   |       | .1722   | .1800  | .2077   |       |
| Cc. N/100 I                         | 5.2  | 13.9   | 12.8    | 15.6  | 1.0   | 13.5   | 11.1    | 15.8  |
| % $\text{Na}_2\text{S}_2\text{O}_3$ |  |        |         |       |   |        |         |       |
| decomposed                          | 66.6   | 10.9   | 18.0    |       | 93.6  | 14.5   | 29.7    |       |
| $\text{H}_2\text{S}$                | 2  | 3      | 4       |       | 2   | 3      | 2       |       |
| Sulphates                           | +  | +      | +       | —     | +   | +      | +       | —     |
| Sporulation                         | 3  | 4      | 4       |       | 3   | 4      | 3       |       |
| Cc. N/10 KOH                        | .9   |        |         | .3    | 1.3   |        |         | .3    |
| Cc. N/10 $\text{H}_2\text{SO}_4$    |  | .1     | .1      |       |   | .1     | .1      |       |

growth have occurred which may be due to sucrose as a better source of carbon, or to the greater total quantity of carbon supplied. These organisms are able to use the thiosulphate with the production of very noticeable quantities of  $\text{H}_2\text{S}$  and sulphates. Using phenolphthalein as an indicator, a production of acidity is indicated for *Aspergillus*, while *Penicillium* and *Botrytis* produce an alkalinity of the solution.

Two different nutrient solutions as employed by Kossowicz and Loew were used in experiments 3 and 4. All salt concentrations are given for 50-cc. cultures. The solution for experiment 3 was as follows:  $\text{Na}_2\text{S}_2\text{O}_3$  M/1, 1.2 cc.;  $\text{KH}_2\text{PO}_4$  M/60, 2.2 cc.;  $(\text{NH}_4)_2\text{HPO}_4$  M/6, 2.2 cc.;  $\text{KNO}_3$  M/4, 4.5 cc.;  $\text{NH}_4\text{NO}_3$  M/1, 1.2 cc.;  $\text{MgCl}_2$  M/60, 2.0 cc.;  $\text{CaCO}_3$  M/100, 1.0 cc.;  $\text{FeCl}_3$  M/1000, 1.0 cc.; dextrose M/1, 6.9 cc.

The solution for experiment 4 was as follows:  $\text{Na}_2\text{S}_2\text{O}_3$  M/1,

4.0 cc.;  $(\text{NH}_4)_2\text{HPO}_4$  M/6, 27.7 cc.;  $\text{KH}_2\text{PO}_4$  M/6, 2.2 cc.;  $\text{MgCl}_2$  M/1, 1.0 cc.;  $\text{CaCO}_3$  M/100, .5 cc.;  $\text{FeCl}_3$  M/1000, .5 cc.; sucrose M/1, 7.3 cc. The length of the experiment was 4 weeks for number 3 and 5 weeks for number 4.

TABLE II

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING SODIUM THIOSULPHATE

| Fungus  | Experiment 3<br>.6 per cent $\text{Na}_2\text{S}_2\text{O}_3$ |        |         |       | Experiment 4<br>2 per cent $\text{Na}_2\text{S}_2\text{O}_3$ |        |         |       |
|---|---|--------|---------|-------|--|--------|---------|-------|
|   | A. nig.   | P. gl. | B. cin. | Check | A. nig.  | P. gl. | B. cin. | Check |
| No. cultures                                      | 5   | 5      | 6       | 1     | 4  | 4      |         | 1     |
| Dry wt. (gms.)                                    | .0448   | .4046  | .2509   |       | .4152  | .6164  | none    |       |
| Cc. N/10 I.                                       | 0   | 1.9    | .9      | 3.0   | 2 1  | 3.8    |         | 8 0   |
| % $\text{Na}_2\text{S}_2\text{O}_3$<br>decomposed | 100   | 36.6   | 70 0    |       | 73.7   | 52.5   |         |       |
| $\text{H}_2\text{S}$                              | —   | —      | —       | —     | 0  | 0      |         | 0     |
| Sulphates   | +   | +      | +       |       | +  | +      |         | —     |
| Sporulation                                       |   |        |         |       | 1  | 3      |         |       |

On the solution employed in experiment 3 *Aspergillus* made very little growth, yet all the thiosulphate had disappeared from the solution. The sulphates determined as  $\text{BaSO}_4$  were so low that other tests were tried to discover the changes which had proceeded, and these seem to indicate the production of a tetrathionate. The tests were made as follows: To the solution in which there was no thiosulphate,  $\text{BaCl}_2$  was added and the sulphate formed was caught in a Gooch crucible. The filtrate was oxidized with bromine and  $\text{BaCl}_2$  again added. A second white precipitate was formed which indicates the production of a polythionate. Qualitative tests with mercurous nitrate gave a yellow precipitate which might be produced by either the tetra- or pentathionate. Potassium hydroxide added to the solution gave no precipitate of sulphur which should be the case if the pentathionate were present. Hence, it appears that the tetrathionate was in the solution.

In the 2 per cent concentration of thiosulphate in the solution as employed in experiment 4, the toxic effects of the thiosulphate

are evident for *Botrytis*. This organism made fairly good growth on the .6 per cent solution of the previous experiment. Since no  $H_2S$  was produced on the 2 per cent thiosulphate solution, an attempt was made to account for all the sulphur by titrating for the thiosulphate, precipitating the sulphate in solution as  $BaSO_4$ ,

TABLE III

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING SODIUM THIOSULPHATE. TIME INTERVAL OF CULTURES, 4 WEEKS

| Fungus                                 | No. of cultures | Dry wt. (gms.) | Cc. N/10 iodine | Per cent $Na_2S_2O_3$ decomposed | $H_2S$ | Sulphate | Sporulation | Cc. KOH N/10 |
|--|-----------------|----------------|-----------------|----------------------------------|--------|----------|-------------|--------------|
| Experiment 5 —5 per cent $Na_2S_2O_3$  |                 |                |                 |                                  |        |          |             |              |
| A. nig.                                | 4               | .3400          | 7.6             | 67.9                             | 2      | +        | 2           | 1.9          |
| P. gl.                                 | 4               | .2502          | 18.5            | 21.9                             | 2      | +        | 4           | 1.4          |
| B. cin.                                |                 | None           |                 |                                  |        |          |             |              |
| Check                                  | 1               |                | 23.7            |                                  |        |          |             | .4           |
| Experiment 6 —10 per cent $Na_2S_2O_3$ |                 |                |                 |                                  |        |          |             |              |
| A. nig.                                | 4               | .2246          | 29.2            | 43.7                             | 3      | +        | 1           | 2.6          |
| P. gl.                                 | 4               | .2578          | 45.2            | 12.9                             | 3      | +        | 3           | .5           |
| B. cin.                                |                 | None           |                 |                                  |        |          |             |              |
| Check                                  |                 |                | 51.9            |                                  |        |          |             |              |
| Experiment 7 —40 per cent $Na_2S_2O_3$ |                 |                |                 |                                  |        |          |             |              |
| A. nig.                                |                 | None           |                 |                                  |        |          |             |              |
| P. gl.                                 | 4               | .5403          | * 19.0          | 7.7                              | 3      | +        | 0           |              |
| B. cin.                                |                 | None           |                 |                                  |        |          |             |              |
| Check                                  |                 |                | * 20.6          |                                  |        |          |             |              |

\* One cc. of the nutrient solution was used for a titration because of the high concentration of the thiosulphate. The average of a number of determinations was taken.

and weighing the free sulphur produced as a precipitate in the flask, but all such efforts gave low results. Raciborski has reported the same difficulty in making quantitative determinations of all the sulphur introduced into the nutrient solution.

The nutrient solution for the experiments in table III was of

the composition given below with only the thiosulphate varied:  $\text{KH}_2\text{PO}_4$  M/60, 2.2 cc.;  $(\text{NH}_4)_2 \text{HPO}_4$  M/6, 2.2 cc.;  $\text{KNO}_3$  M/4, 4.7 cc.;  $\text{NH}_4\text{NO}_3$  M/1, 1.2 cc.;  $\text{MgCl}_2$  M/60, 2.8 cc.;  $\text{CaCO}_3$  M/100, 0.5 cc.;  $\text{FeCl}_3$  M/1000, 0.5 cc.; dextrose M/1, 6.9 cc. Of the M/1  $\text{Na}_2\text{S}_2\text{O}_3$ , 10.1 cc. are used in the 5 per cent solution, 20.2 cc. in the 10 per cent solution, and 26.8 cc. of a 3/M  $\text{Na}_2\text{S}_2\text{O}_3$  in the 40 per cent solution. In every case sufficient water is added to make the volume of each culture 50 cc.

In the series of increasing thiosulphate concentrations made up as 5, 10, and 40 per cent solutions, the growth of *Penicillium* was successively greater, as was the production of sulphates and  $\text{H}_2\text{S}$ , though sporulation was prevented in the solution of highest concentration. In the nutrient solutions employed, the inhibition of growth for *Botrytis* in the presence of the thiosulphate occurs between .6 and 2 per cent, for *Aspergillus* between 10 and 40 per

TABLE IV

GROWTH OF CERTAIN FUNGI ON MEDIA CONTAINING POTASSIUM THIOCYANATE AND MAGNESIUM SULPHATE. TIME INTERVAL OF CULTURES, 27 DAYS

| Fungus               | Experiment 8<br>.2 per cent KCNS |        |         |       | Experiment 9<br>KCNS replaced by $\text{MgSO}_4$ |        |         |       |
|----------------------|----------------------------------|--------|---------|-------|--|--------|---------|-------|
|                      | A. nig.                          | P. gl. | B. cin. | Check | A. nig.  | P. gl. | B. cin. | Check |
| No. cultures         | 6                                | 5      | 5       |       | 5  | 5      | 4       |       |
| Dry wt. (gms.)       | .3512                            | .1602  | .4155   |       | .9212  | .4234  | 1.0766  |       |
| Sporulation          | 1                                | 0      | 0       |       | 4  | 4      | 4       |       |
| $\text{H}_2\text{S}$ | 0                                | 0      | 0       | 0     | 0  | 0      | 0       | 0     |

cent, while *Penicillium* grows very well on the 40 per cent solution. These statements refer to solutions in which the hydrogen-ion concentration was unknown. In the later experiments it will be shown that the toxic concentration of a salt in solution may depend to some extent on the hydrogen-ion concentration of the medium.

The nutrient solution employed was  $\text{NH}_4\text{NO}_3$  M/1, 6.2 cc.;  $\text{KH}_2\text{PO}_4$  M/1, 2.5 cc.;  $\text{FeCl}_3$  M/1000, 0.5 cc.; sucrose M/1, 7.3 cc. in each solution, with the magnesium and sulphur supplied in experiment 8 as 1 cc. each of M/1  $\text{MgCl}_2$  and KCNS. In ex-

periment 9 the KCNS and  $MgCl_2$  were omitted and the magnesium and sulphur were supplied in 1 cc. of M/1  $MgSO_4$ . The potassium and chlorine content were kept practically the same by adding 1 cc. of M/1 KCl. Both growth and fructification have been greatly increased where sulphur is supplied as  $MgSO_4$  instead of KCNS.

TABLE V

GROWTH OF CERTAIN FUNGI ON MEDIA CONTAINING POTASSIUM THIOCYANATE AND AMMONIUM THIOCYANATE. TIME INTERVAL OF CULTURES, 4 WEEKS

| Fungus         | Experiment 10<br>1 per cent KCNS |        |         |       | Experiment 11<br>1 per cent $NH_4CNS$ |        |         |       |
|----------------|----------------------------------|--------|---------|-------|---------------------------------------|--------|---------|-------|
|                | A. nig.                          | P. gl. | B. cin. | Check | A. nig.                               | P. gl. | B. cin. | Check |
| No. cultures   | 2                                | 2      | 2       |       | 2                                     | 2      | 2       |       |
| Dry wt. (gms.) | .1313                            | .0159  | .0238   |       | .0445                                 | .0125  | .0250   |       |
| $H_2S$         | 1                                | 1      | 3       | 0     | 1                                     | 1      | 3       | 0     |
| Sporulation    | 0                                | 0      | 0       |       | 0                                     | 0      | 0       |       |

Nutrient solutions as employed by Kossowicz and Gröller were used and are, for experiment 10, KCNS 2/M, 2.5 cc.;  $KNO_3$  M/1, 2.4 cc.;  $NH_4NO_3$  M/1, 3.1 cc.;  $KH_2PO_4$  M/6, 2.2 cc.;  $MgSO_4$  M/3, 3.0 cc.; dextrose M/1, 6.9 cc. The salts in the solution for experiment 11 were  $NH_4CNS$  M/1, 6.6 cc.;  $KH_2PO_4$  M/6, 2.2 cc.;  $MgCl_2$  M/60, 7.4 cc.;  $CaCO_3$  M/100, 1.0 cc.;  $FeCl_3$  M/1000, 1.0 cc.; dextrose M/1, 5.6 cc. In the solutions employed, 1 per cent of both KCNS and  $NH_4CNS$  strongly inhibits the growth of the 3 organisms.

The nutrient solution for the experiments 12, 14, and 15 of tables VI and VII was the same except for the source of sulphur. The constituents of the solution were  $NH_4NO_3$  M/1, 6.2 cc.;  $KH_2PO_4$  M/1, 2.5 cc.;  $MgCl_2$  M/1, 1.0 cc.;  $FeCl_3$  M/1000, .5 cc.; dextrose M/1, 6.9 cc. The sulphur compounds were added in such quantities that the sulphur content of the solutions in the three experiments mentioned would be the same. Twenty-five cc. of M/50  $K_2S_2O_8$  were added per flask in experiment 12, 1 cc. of M/1<sup>0</sup> KSH in experiment 14, and 1 cc. of M/1  $MnSO_4$  in experiment 15. The solution to which equivalent amounts of

KHSO<sub>3</sub> were added showed no growth whatever, probably due to the high acidity as found by titration. The good growth obtained on the KHSO<sub>3</sub> in experiment 13 was in a solution where this salt was used as a source of both the potassium and sulphur with only a slight acidity produced. The solution was KHSO<sub>3</sub> M/1, 2.5 cc.; (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> M/1, 6.2 cc.; MgCl<sub>2</sub> M/1, 1.0 cc.;

TABLE VI

GROWTH OF CERTAIN FUNGI ON MEDIA CONTAINING POTASSIUM PERSULPHATE AND POTASSIUM BISULPHITE. TIME INTERVAL OF CULTURES, 4 WEEKS

| Fungus           | Experiment 12<br>.3 per cent K <sub>2</sub> S <sub>2</sub> O <sub>8</sub> |        |         |       | Experiment 13<br>.6 per cent KHSO <sub>3</sub> |        |         |       |
|------------------|---|--------|---------|-------|--|--------|---------|-------|
|                  | A. nig.   | P. gl. | B. cin. | Check | A. nig.  | P. gl. | B. cin. | Check |
| No. cultures     | 3   |        | 3       |       | 3  | 3      | 3       |       |
| Dry wt. (gms.)   | .4183   | None   | .1699   |       | .2704  | .2670  | .2202   |       |
| H <sub>2</sub> S | 0   | 0      | 0       | 0     | 0  | 0      | 3       | 0     |
| Sporulation      | 1   |        | 0       |       | 4  | 3      | 0       |       |

TABLE VII

GROWTH OF CERTAIN FUNGI ON MEDIA CONTAINING POTASSIUM HYDRO SULPHITE AND MANGANESE SULPHATE. TIME INTERVAL OF CULTURES, 4 WEEKS

| Fungus           | Experiment 14<br>.15 per cent KSH |        |         |       | Experiment 15<br>.3 per cent MnSO <sub>4</sub> |        |         |       |
|------------------|-----------------------------------|--------|---------|-------|--|--------|---------|-------|
|                  | A. nig.                           | P. gl. | B. cin. | Check | A. nig.  | P. gl. | B. cin. | Check |
| No. cultures     | 2                                 | 2      | 2       |       | 2  | 2      | 2       |       |
| Dry wt. (gms.)   | .3208                             | .1039  | .2684   |       | .4508  | .3936  | .4049   |       |
| H <sub>2</sub> S | 0                                 | 0      | 0       | 0     | 0  | 0      | 0       | 0     |
| Sporulation      | 4                                 | 1      | 4       |       | 4  | 4      | 4       |       |

FeCl<sub>3</sub> M/1000, .5 cc.; dextrose M/1, 6.9 cc. K<sub>2</sub>S was also used as a source of sulphur with very meagre growth obtained. Under the conditions of these 4 experiments, MnSO<sub>4</sub> is the most favorable of the salts for the growth of all the organisms. The only case of complete inhibition of growth was for *Penicillium* on K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>.

## B. THE USE OF THIOSULPHATE IN RELATION TO HYDROGEN-ION CONCENTRATION

The experimental work as reported in the first part of this paper failed to show any direct relation between the use of thio-sulphate and the growth produced, though there were indications

TABLE VIII

AMOUNTS OF ACID OR ALKALI NECESSARY TO PRODUCE AN INITIAL  $P_H$  AS INDICATED

| Cc. N/5 HCl<br>per 50 cc.<br>final volume | Cc. N/5 NaOH<br>per 50 cc.<br>final volume | Initial $P_H$ |
|---|--|---------------|
| 2.0                                       |  | 3.0           |
| .3  |  | 4.2           |
| 0   | 0  | 4.5           |
|   | 5.0  | 5.9           |
|   | 15.0                                       | 7.1           |

TABLE IX

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING .6 PER CENT SODIUM THIOSULPHATE. INITIAL  $P_H$  4.2. EXPERIMENT 16

| Fungus   | Days | Dry wt.<br>(gms.) | Final<br>$P_H$ | Cc.<br>N/100<br>iodine | Per cent<br>$Na_2S_2O_3$<br>decomposed | $H_2S$ | Sul-<br>phate | Sporu-<br>lation |
|----------|------|-------------------|----------------|------------------------|--|--------|---------------|------------------|
| A. nig.  | 5    | .0719             | 3.8            | 17.4                   | 13.8                                   | 2      | —             | 0                |
|          | 7    | .1459             | 3.0            | 13.5                   | 33.1                                   | 2      | +             | 0                |
|          | 10   | .1553             | 2.0            | 11.6                   | 42.5                                   | 2      | +             | 0                |
|          | 13   | .1706             | 3.2            | 9.5                    | 52.9                                   | 2      | +             | 0                |
| P. cycl. | 5    | .3035             | 3.1            | 12.6                   | 37.6                                   | 1      | +             | 0                |
|          | 7    | .5938             | 3.0            | 7.9                    | 60.9                                   | 2      | +             | 0                |
|          | 10   | .7374             | 3.2            | 5.3                    | 73.7                                   | 2      | +             | 1                |
|          | 13   | .6628             | 5.6            | 6.2                    | 69.3                                   | 3      | +             | 4                |
| B. cin.  | 5    | .0160             | 3.9            | 17.2                   | 14.8                                   | 2      | —             | 0                |
|          | 7    | .0416             | 3.9            | 17.1                   | 15.3                                   | 2      | —             | 0                |
|          | 10   | .1166             | 3.6            | 16.7                   | 17.3                                   | 2      | +             | 0                |
|          | 13   | .1190             | 3.7            | 13.1                   | 35.1                                   | 2      | +             | 0                |
| Control  |      |                   | 4.2            | 20.2                   |  | 0      | —             |                  |



from the tests with litmus paper and the determinations of titratable acidity that the reaction of the medium might be a factor of considerable importance. In the experiments with some of the salts other than the thiosulphate, it was also realized that rather

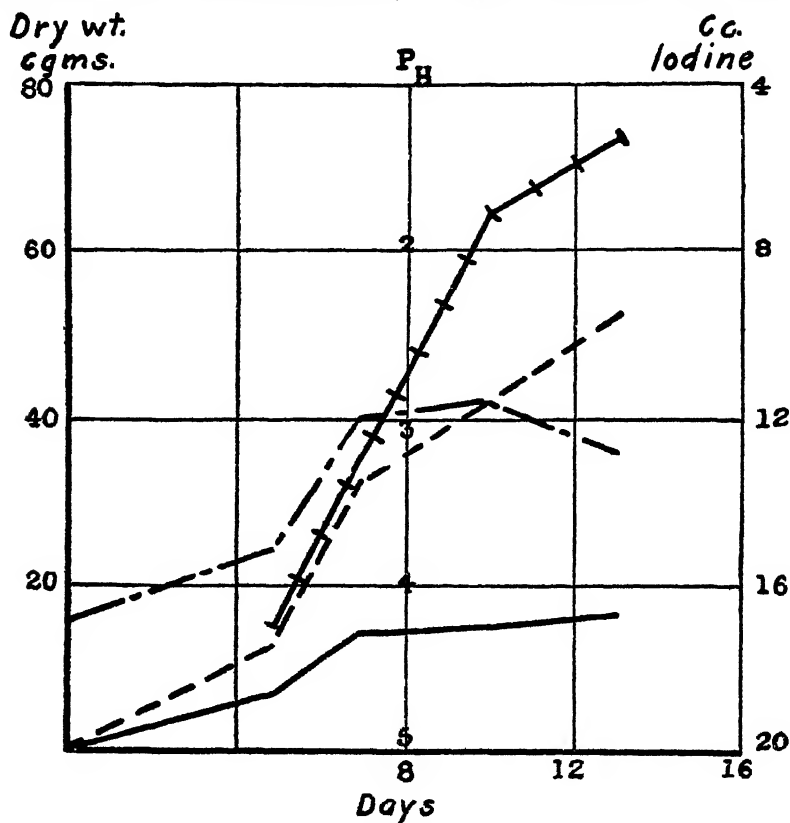


Fig. 1. *Aspergillus niger* on solution of initial  $P_H$  4.2 containing thiosulphate.

— dry weight.  
 - - - - - thiosulphate decomposed expressed as cc.  
           N/100 iodine.  
 - . - . - hydrogen ion concentration.  
 - + + + - ratio  $\frac{\text{thiosulphate decomposed}}{\text{growth}}$ .  
 (The legend above holds for figures 1-3 and 7-15.)

acid solutions were produced in some cases, and the effect of this factor on the "toxic concentration" of the salt employed was entirely problematical. In this series of experiments the thio-

sulphate content was held constant at practically a .6 per cent solution, while the hydrogen-ion concentration was varied from an initial  $P_H$  of 3.0 to 7.1. Increases in the hydrogen-ion concentration of the original medium were obtained by the addition

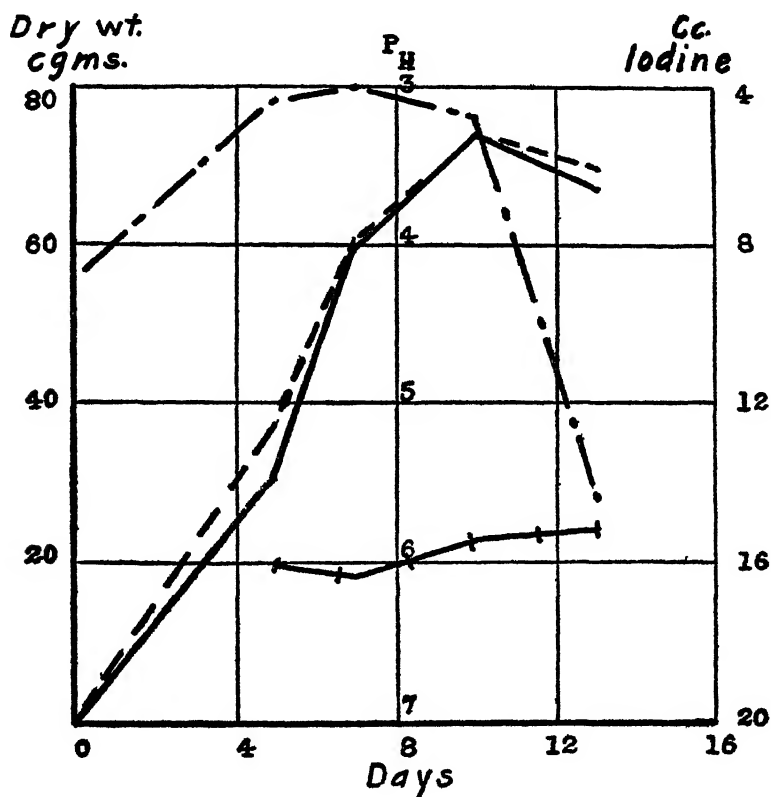


Fig. 2. *Penicillium cyclopium* on solution of initial  $P_H$  4.2 containing thiosulphate.

of  $N/5$  HCl, while the decreases were obtained by the use of  $N/5$  NaOH. If either of these compounds be added before sterilization, a caramelization and consequently a decided change in the medium occurs, so that the following procedure was adopted. The solution was mixed *en masse* for a particular series and (50—x) cc. pipetted into flasks of 120 cc. capacity which were then sterilized in an autoclave at 15 pounds pressure for 20 minutes. After cooling, x cc. of sterile acid or alkali were added under sterile conditions to each flask which was then allowed to

stand for 24 hours so that a state of equilibrium would be attained. By this procedure the final volume of the solution was 50 cc., and the concentration of the salts was comparable to that of the original solution.

The successive increases in growth, decreases in the amount of

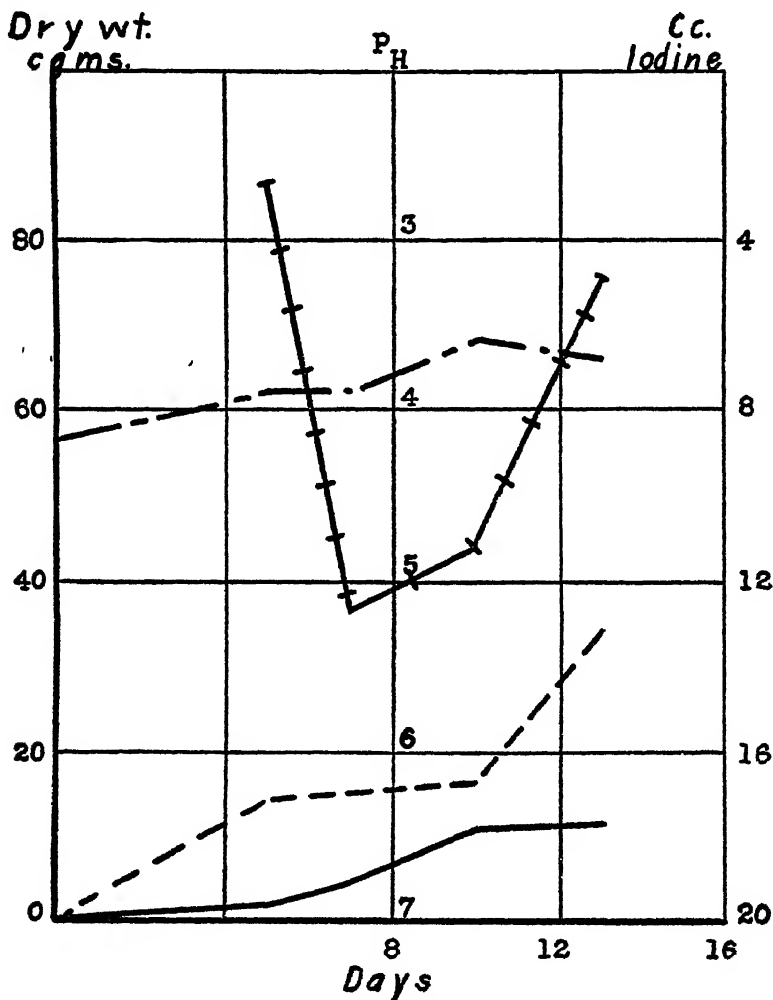


Fig. 3. *Botrytis cinerea* on solution of initial  $P_H$  4.2 containing thiosulphate.

thiosulphate present, and changes in the reaction were determined for each series of cultures.

The composition of the medium was as follows:  $\text{NH}_4\text{NO}_3$  M/1, 6.2 cc.;  $\text{KH}_2\text{PO}_4$  M/1, 2.5 cc.;  $\text{MgCl}_2$  M/1, 1.0 cc.;  $\text{Na}_2\text{S}_2\text{O}_3$  M/1, 1.2 cc.; sucrose M/1, 7.3 cc.;  $\text{FeCl}_3$  M/1000, .5 cc.; water plus acid or alkali sufficient to make 50 cc.

The addition of NaOH caused some precipitation in the medium but this was disregarded.

*Experiment 16.*—In following the successive changes in the cultures, quantitative determinations were made of growth, changes in  $P_H$ , and the thiosulphate content of the solution, qualitative tests for  $\text{H}_2\text{S}$  and sulphates, and observations on the sporulation of the cultures. The relations between growth, hydrogen ion, and the consumption of thiosulphate are more clearly shown in figs. 1, 2, and 3. Both *Aspergillus* and *Botrytis* show a rather meagre growth on this acid solution, while *Penicillium* grows very well, surpassing this growth in only one other solution, that of  $P_H$  4.5. A series at  $P_H$  3.0 was inoculated but no growth was obtained with any of the organisms.

If any direct relation exists between the decomposition of thiosulphate and the resulting growth, the ratio of thiosulphate to growth would be a constant and would appear as a straight line when plotted on coördinate paper. If the possibilities of experimental error are considered, there appears to be such a straight-line relationship in 8 of the 12 cases determined and represented in figs. 4, 5, and 6. *Penicillium* seems to exhibit the direct relationship between the decomposition of thiosulphate and growth in the series of cultures at initial  $P_H$  4.2, while *Aspergillus* and *Botrytis* exhibit the most extreme variations. The hydrogen-ion concentration of the solution does not appear to be the factor involved in these variations, as the hydrogen-ion concentration produced by *Botrytis* was lowest during most of the experiment and yet this fungus was the most inefficient user or decomposer of thiosulphate when dry weight is considered as a criterion of efficiency. In some of the other series of experiments it may be seen that a similar low production of acidity may not similarly affect the ratio curve. A point of interest is the decided reversion of the reaction in the solution supporting the growth of *Penicillium*. This decided reversion of the reaction occurred<sup>9</sup> between the tenth and thirteenth days at the period when the mycelium suddenly produced a large number of spores.

Such a reversion of the reaction at the initiation of the fruiting stage will also be noted for *Penicillium* in the series at  $P_H$  7.1. However, no such noticeable reversions of the reaction occurred

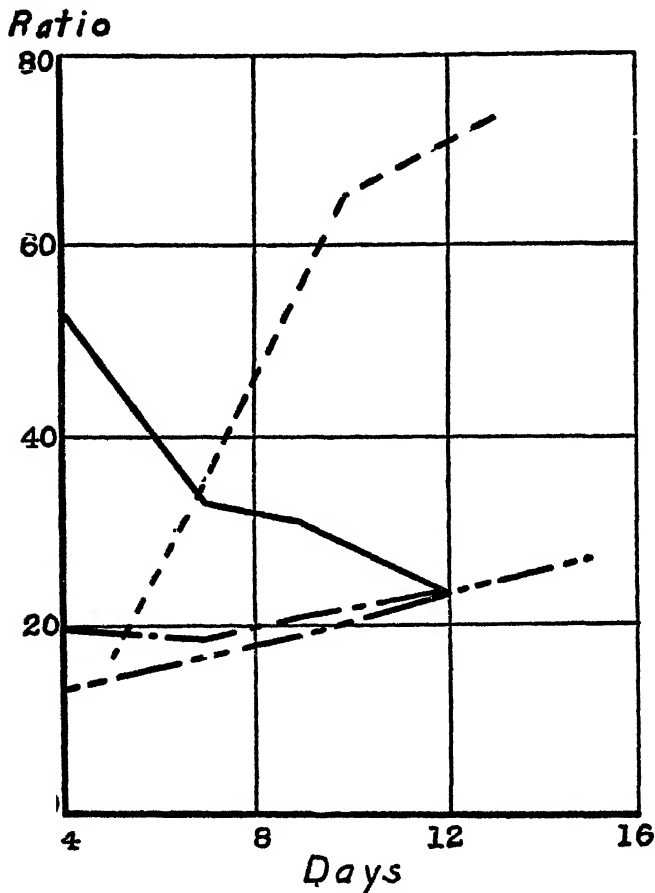


Fig. 4. *Aspergillus niger*, ratio  $\frac{\text{thiosulphate decomposed}}{\text{growth}}$ .

----- initial  $P_H$  4.2.

————— initial  $P_H$  4.5.

- - - - - initial  $P_H$  5.9.

— · — · — initial  $P_H$  7.1.

(This legend holds for figs. 4-6.)

with *Aspergillus* or *Botrytis* at any stage of growth on the solutions containing the thiosulphate.

Two products of the decomposition of the thiosulphate are

H<sub>2</sub>S and sulphates. The control flasks, though remaining sterile throughout, had given rise to H<sub>2</sub>S after 5 days.

TABLE X

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING .6 PER CENT SODIUM THIOSULPHATE. INITIAL P<sub>H</sub> 4.5. EXPERIMENT 17

| Fungus   | Days | Dry wt.<br>(gms.) | Final<br>P <sub>H</sub> | Cc.<br>N/100<br>iodine | Per cent<br>Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub><br>decomposed | H <sub>2</sub> S | Sul-<br>phate | Sporu-<br>lation |
|----------|------|-------------------|-------------------------|------------------------|---|------------------|---------------|------------------|
| A. nig.  | 4    | .1251             | 3.1                     | 14.8                   | 34.5  | 0                | +             | 0                |
|          | 7    | .5258             | 2.1                     | 4.4                    | 80.5  | 0                | +             | 3                |
|          | 9    | .6598             | 1.8                     | 1.5                    | 93.3  | 0                | +             | 3                |
|          | 12   | .9763             | 1.7                     | .3                     | 98.7  | 0                | +             | 3                |
|          | 15   | .7578             | 1.8                     | .2                     | 99.1  | 0                | +             | 4                |
|          | 18   | .7619             | 1.9                     | 0                      | 100   | 0                | +             | 4                |
| P. cycl. | 4    | .0483             | 4.1                     | 20.6                   | 8.8   | 1                | —             | 0                |
|          | 7    | .2483             | 3.8                     | 17.8                   | 21.2  | 1                | +             | 0                |
|          | 9    | .3961             | 3.4                     | 15.4                   | 31.5  | 1                | +             | 0                |
|          | 12   | .5143             | 3.6                     | 12.4                   | 41.3  | 1                | +             | 0                |
|          | 15   | .7755             | 2.9                     | 5.6                    | 75.2  | 1                | +             | 0                |
|          | 18   | .6751             | 2.0                     | 5.4                    | 76.0  | 1                | +             | 1                |
| B. cin.  | 4    | .0643             | 3.8                     | 19.5                   | 13.7  | 1                | +             | 0                |
|          | 7    | *.3002            | 3.0                     | 10.7                   | 52.6  | 3                | +             | 0                |
|          | 9    | .2462             | 3.1                     | 10.8                   | 52.2  | 3                | +             | 2                |
|          | 12   | .4148             | 3.0                     | 3.8                    | 83.1  | 3                | +             | 3                |
|          | 15   | .7755             | 2.9                     | 1.1                    | 95.1  | 3                | +             | 4                |
| Control  |      |                   | 4.5                     | 22.6                   |   | † 1              | 0             |                  |

\* 2 cultures only.

† Slight after 15 days.

*Experiment 17.*—The solution for experiment 17 was the original solution without the addition of acid or alkali. Notwithstanding careful technique in the preparation of the solution and the use of the best chemicals obtainable, the initial P<sub>H</sub> of the solution varied as much as .4 P<sub>H</sub> from time to time. More generally the reaction of the freshly prepared solution was P<sub>H</sub> 4.9.

The increase in growth of *Aspergillus* and *Botrytis* over that obtained in the previous solution was marked, while *Penicillium*

though showing a slightly greater growth, required a third longer time to attain this. As in the former experiment, *Botrytis* decomposed a greater quantity of thiosulphate per unit weight than

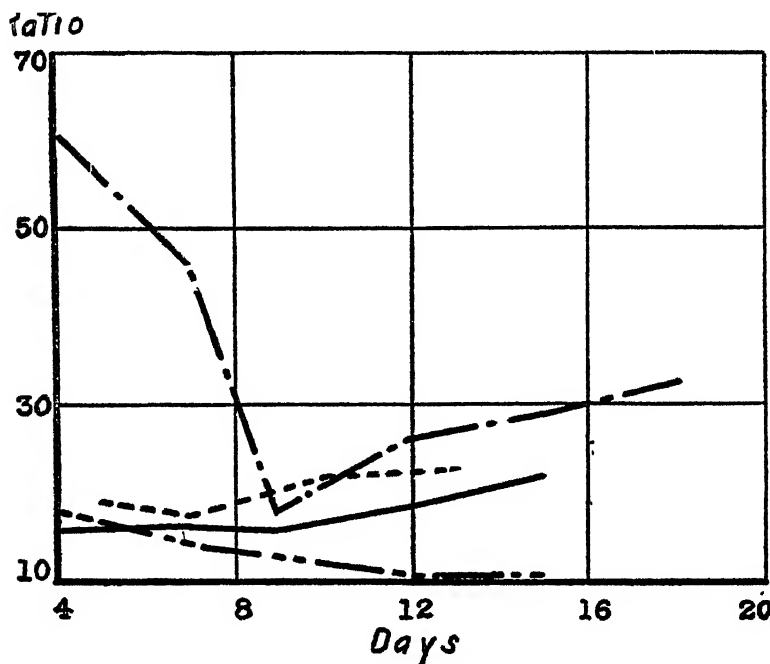


Fig. 5. *Penicillium cyclopium*, ratio  $\frac{\text{thiosulphate decomposed}}{\text{growth}}$ .

the other organisms and produced a greater darkening of the lead-paper, indicating a greater production of  $\text{H}_2\text{S}$ . *Aspergillus*, though producing an acidity of the solution amounting to  $\text{P}_\text{H}$  1.7 and finally decomposing all the thiosulphate, failed to produce any  $\text{H}_2\text{S}$ . This fact is not in agreement with the idea of some earlier workers that the acidity produced in the solution might be responsible for the separation of free sulphur and the production of  $\text{H}_2\text{S}$ . The control flask produced a trace of  $\text{H}_2\text{S}$  after the fifteenth day but no sulphates were present. The results are shown more clearly in figs. 7, 8, and 9.

*Experiment 18.*—The chief results as presented in table xi are shown graphically in figs. 10, 11, and 12. *Aspergillus* and *Botrytis* made further increases in growth over the two previous

TABLE XI

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING .6 PER CENT SODIUM THIOSULPHATE. INITIAL  $P_H$  5.9. EXPERIMENT 18

| Fungus   | Days | Dry wt.<br>(gms.) | Final<br>$P_H$ | Cc.<br>N/100<br>iodine | Per cent<br>$Na_2S_2O_3$<br>decomposed | $H_2S$ | Sul-<br>phate | Sporu-<br>lation |
|----------|------|-------------------|----------------|------------------------|--|--------|---------------|------------------|
| A. nig.  | 4    | * .7956           | 2.1            | 5.5                    | 74.3                                   | 1      | +             | 0                |
|          | 7    | * 1.1256          | 1.7            | 0.6                    | 97.2                                   | 1      | +             | 1                |
|          | 9    | * .9742           | 2.0            | 0.4                    | 98.1                                   | 1      | +             | 1                |
|          | 12   | * .9248           | 2.2            | 0.3                    | 98.6                                   | 1      | +             | 2                |
|          | 15   | * .8836           | 2.2            | 0.2                    | 99.0                                   | 1      | +             | 2                |
|          | 18   | * 1.1650          | 2.3            | 0                      | 100                                    | 1      | +             | 3                |
| P. cycl. | 4    | .0735             | 5.6            | 16.4                   | 23.3                                   | 1      | +             | 0                |
|          | 7    | .1855             | 4.9            | 12.9                   | 39.7                                   | 1      | +             | 0                |
|          | 9    | .4904             | 3.8            | 12.4                   | 42.0                                   | 2      | +             | 0                |
|          | 12   | .5166             | 3.8            | 7.6                    | 64.4                                   | 2      | +             | 0                |
|          | 15   | .5671             | 4.1            | 5.2                    | 74.7                                   | 2      | +             | 0                |
|          | 18   | .5771             | 4.2            | 2.5                    | 88.3                                   | 3      | +             | 1                |
| B. cin.  | 4    | * .1203           | 5.4            | 19.3                   | 9.8                                    | 1      | +             | 0                |
|          | 7    | .3526             | 3.5            | 12.4                   | 42.0                                   | 1      | +             | 2                |
|          | 9    | .6415             | 3.5            | 8.1                    | 62.1                                   | 1      | +             | 2                |
|          | 12   | 1.0472            | 2.8            | 4.0                    | 81.3                                   | 1      | +             | 4                |
|          | 15   | .9991             | 2.6            | 3.4                    | 84.1                                   | 1      | +             | 4                |
|          | 29   | .8423             | 3.4            | 0                      | 100                                    | 1      | +             | 4                |
| Control  |      |                   | 5.9            | 21.4                   |  | 0      | +             |                  |

\* 2 cultures only.

solutions, *Aspergillus* attaining this growth very rapidly with a change in the reaction to  $P_H$  1.7 on the seventh day. The rapid increase of growth of *Aspergillus* to the seventh day followed by a decline and a second maximum on the eighteenth day when the thiosulphate had disappeared did not occur in other cultures, so that the significance of this is not known. The unit of dry weight per unit of thiosulphate decomposed was practically the same for *Aspergillus* and *Botrytis*, with *Penicillium* exhibiting a variable ratio as shown in fig. 5. *Penicillium* was the strongest producer of  $H_2S$  though the total quantity of thiosulphate decomposed was less than for the other organisms employed.



*Experiment 19.*—The results as presented in table XII are shown graphically in figs. 13, 14, and 15. All the fungi made a good growth on this solution, *Aspergillus* growing rapidly, with *Botrytis* appearing so slowly that no determinations were obtain-

TABLE XII

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING .6 PER CENT SODIUM THIOSULPHATE. INITIAL  $P_H$  7.1. EXPERIMENT 19

| Fungus   | Days | Dry wt.<br>(gms.) | Final<br>$P_H$ | Cc.<br>N/100<br>iodine | Per cent<br>$Na_2S_2O_3$<br>decomposed | $H_2S$ | Sul-<br>phate | Sporu-<br>lation |
|----------|------|-------------------|----------------|------------------------|--|--------|---------------|------------------|
| A. nig.  | 4    | .3221             | 3.4            | 16.9                   | 23.5                                   | 0      | +             | 1                |
|          | 7    | .6840             | 2.9            | 10.1                   | 54.3                                   | 0      | +             | 2                |
|          | 9    | .9156             | 2.9            | 5.1                    | 76.9                                   | 0      | +             | 3                |
|          | 12   | .9168             | 2.9            | 2.0                    | 90.9                                   | 1      | +             | 4                |
|          | 15   | .8207             | 2.9            | 1.1                    | 95.0                                   | 1      | +             | 4                |
| P. cycl. | 4    | .1864             | 4.4            | 17.6                   | 20.3                                   | 0      | +             | 3                |
|          | 7    | .3595             | 4.9            | 16.3                   | 26.2                                   | 0      | +             | 3                |
|          | 9    | .5026             | 5.1            | 16.3                   | 26.2                                   | 0      | +             | 3                |
|          | 12   | .6914             | 5.3            | 15.1                   | 31.6                                   | 0      | +             | 3                |
|          | 15   | .6602             | 5.8            | 15.9                   | 28.0                                   | 0      | +             | 4                |
| B. cin.  | 7    | .1352             | 6.1            | 19.0                   | 14.0                                   | 0      | +             | 4                |
|          | 9    | .2687             | 5.4            | 15.7                   | 28.9                                   | 0      | +             | 4                |
|          | 12   | .4143             | 5.2            | 12.8                   | 42.0                                   | 0      | +             | 4                |
|          | 15   | .6480             | 4.9            | 9.5                    | 57.0                                   | 1      | +             | 4                |
|          | 18   | .7861             | 4.7            | 5.6                    | 74.6                                   | 1      | +             | 4                |
| Control  | —    | —                 | 7.1            | 22.1                   | —                                      | 0      | +             | —                |

able at the end of 4 days. Webb ('19) has found the alkaline limit for the spores of *Botrytis* to be near the neutral point,  $P_H$  7, and here we apparently have a case of retarded germination and growth until the change in reaction of the substratum has reached the favorable range of acidity, where a rapid increase in growth can occur. This will appear from an examination of table XIII showing the successive increases in growth of each organism in this solution. *Aspergillus* usually makes relatively large and rapid increases in growth, *Botrytis* usually attains the largest

successive increases at a later period, while *Penicillium* generally displays an intermediate condition. The ratio of the thiosulphate decomposed per unit of dry weight produced is about the

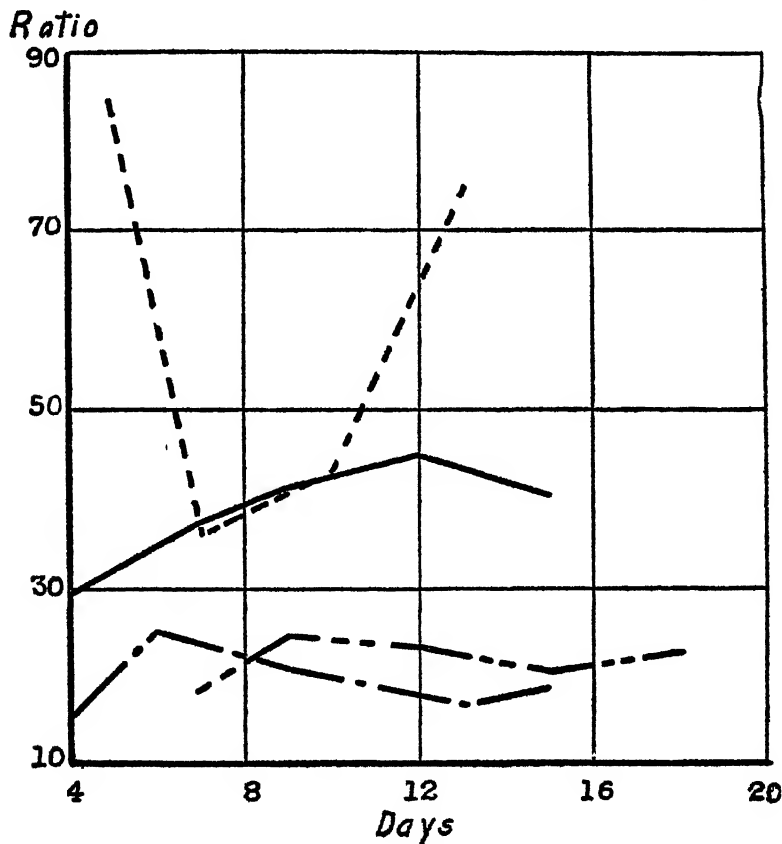


Fig. 6. *Botrytis cinerea*, ratio  $\frac{\text{thiosulphate decomposed}}{\text{growth}}$ .

same for *Aspergillus* and *Botrytis*, with *Penicillium* showing a more economical decomposition of the thiosulphate than the other fungi. No  $\text{H}_2\text{S}$  was produced by *Penicillium*, and there was only a slight production of this compound after the twelfth day by *Aspergillus* and after the fifteenth day by *Botrytis*. A very interesting reversion of the reaction occurred in the cultures of *Penicillium* after the first determination at 4 days, and this may have begun even earlier. No tests were made for sugar in

these cultures, though later experiments in this report will show that *Penicillium* may cause a reversion of the reaction before the sugar has disappeared.

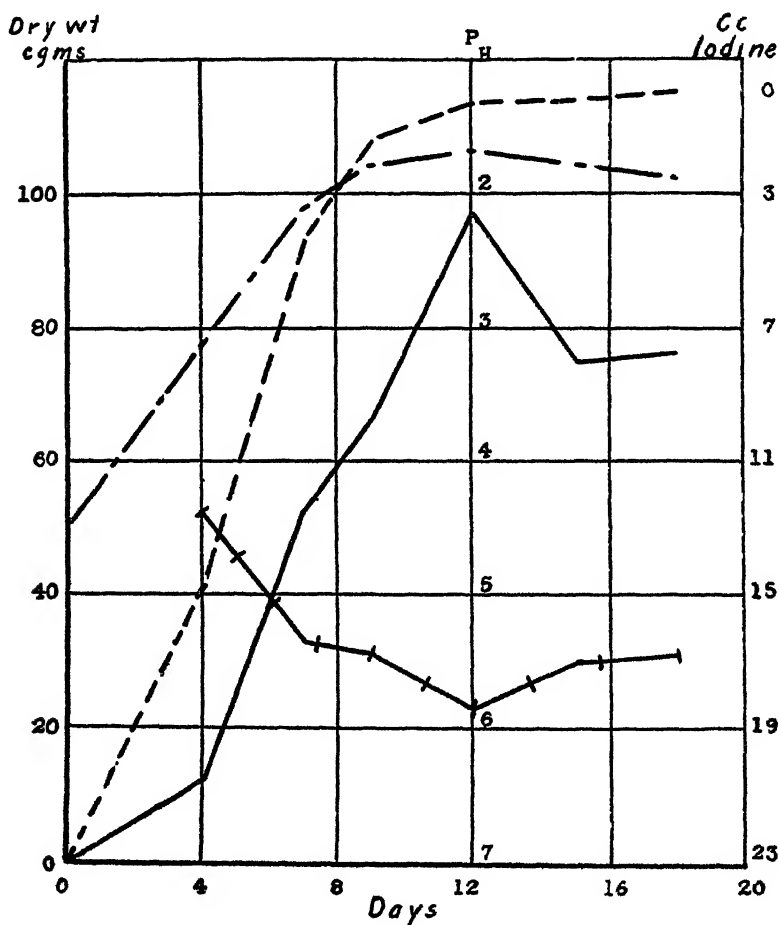


Fig. 7. *Aspergillus niger* on solution of initial  $P_H$  4.5 containing thiosulphate.

#### C. GROWTH AND HYDROGEN-ION CONCENTRATION WITH $MgSO_4$ SUBSTITUTED FOR $Na_2S_2O_3$ AS A SOURCE OF SULPHUR

The basic solution in which equi-molecular weight substitutions as  $Na_2S_2O_3$  were employed in the 4 preceding experiments was now used with  $MgSO_4$  as the source of sulphur. To keep the

TABLE XIII

SUCCESSIVE INCREASES IN DRY WEIGHT ON THE SOLUTION WITH AN INITIAL  $P_H$  OF 7.1

| Fungus   | Days  |       |       |       |       |       |
|----------|-------|-------|-------|-------|-------|-------|
|          | 4     | 7     | 9     | 12    | 15    | 18    |
| A. nig.  | .3221 | .3619 | .2316 | .0012 | —     | —     |
| P. cycl. | .1864 | .1731 | .1431 | .1838 | —     | —     |
| B. cin.  | —     | .1352 | .1335 | .1456 | .2337 | .1381 |

TABLE XIV

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING MAGNESIUM SULPHATE. INITIAL  $P_H$  4.1. EXPERIMENT 20

| Fungus   | Days | Dry wt.<br>(gms ) | Final<br>$P_H$ | Sugar  | Sulphates | H <sub>2</sub> S | Sporulation |
|----------|------|-------------------|----------------|--------|-----------|------------------|-------------|
| A. nig.  | 4    | .8569             | 1.6            | +      | +         | 0                | 0           |
|          | 7    | 1.0369            | 1.5            | 0      | +         | 0                | 1           |
|          | 9    | .9812             | 1.7            | 0      | +         | 0                | 1           |
|          | 12   | .9186             | 1.7            | 0      | +         | 0                | 1           |
|          | 15   | .8723             | 1.9            | 0      | +         | 0                | 1           |
|          | 18   | .8423             | 1.9            | 0      | +         | 0                | 1           |
| P. cycl. | 4    | .3232             | 3.7            | +      | +         | 0                | 1           |
|          | 7    | .8624             | 3.7            | +      | +         | 0                | 3           |
|          | 9    | .9446             | 4.2            | +      | +         | 0                | 3           |
|          | 12   | 1.1722            | 4.2            | Slight | +         | 0                | 3           |
|          | 15   | .9807             | 4.6            | Slight | +         | 0                | 4           |
|          | 18   | .9344             | 5.0            | 0      | +         | 0                | 4           |
| B. cin.  | 4    | .1915             | 2.9            | +      | +         | 0                | 3           |
|          | 7    | .5791             | 2.9            | +      | +         | 0                | 3           |
|          | 9    | .7224             | 2.4            | +      | +         | 0                | 3           |
|          | 12   | .8643             | 2.2            | +      | +         | 0                | 3           |
|          | 15   | 1.0060            | 2.4            | +      | +         | 0                | 3           |
|          | 18   | 1.0123            | 2.4            | +      | +         | 0                | 4           |
| Control  |      |                   | 4.1            | +      | +         | 0                |             |

balance of sodium and chlorine ions as nearly as possible that of the former solution where  $\text{Na}_2\text{S}_2\text{O}_3$  and  $\text{MgCl}_2$  were used, a sufficient amount of  $\text{NaCl}$  was added to maintain the balance of sodium and change the balance of chlorine only very slightly.

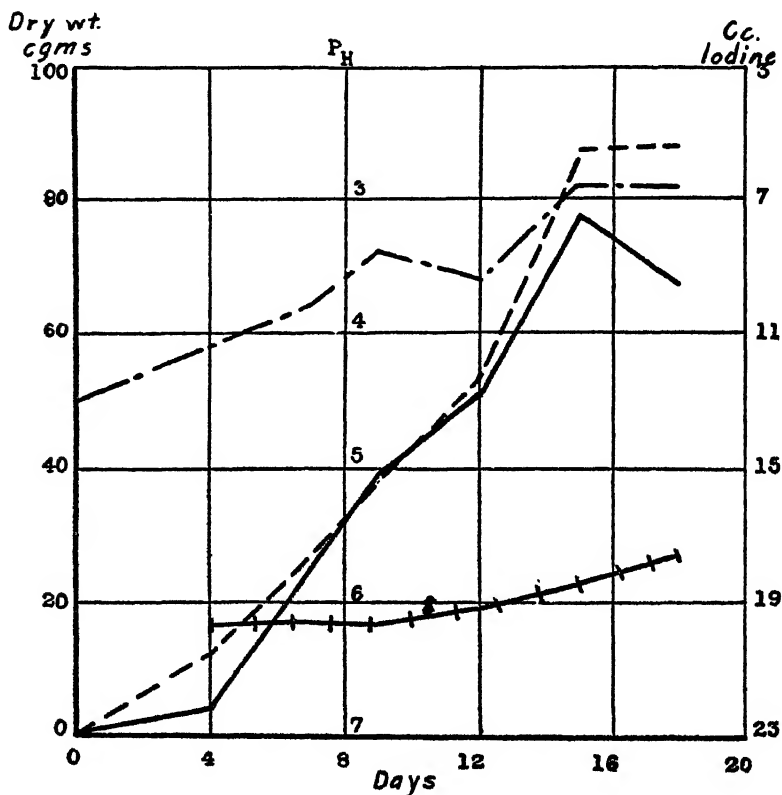


Fig. 8. *Penicillium cyclopium* on solution of initial  $P_H$  containing thiosulphate.

The modified Pfeffer's solution in which the substitutions were made is known to be a favorable solution for the growth of many fungi, hence this series of cultures was grown to determine if there had been any retarding effect of the thiosulphate in solutions of the same initial  $P_H$ , as well as the relative changes in the hydrogen-ion concentration, the production of  $\text{H}_2\text{S}$ , and the reversions in reaction in relation to the disappearance of the sugar as determined by qualitative tests with Fehling's solution. The

composition of the solution was as follows:  $\text{NH}_4\text{NO}_3$  M/1, 6.2 cc.;  $\text{NaCl}$  M/1, 2.4 cc.;  $\text{KH}_2\text{PO}_4$  M/1, 2.5 cc.;  $\text{MgSO}_4$  M/1, 1.0 cc.;  $\text{FeCl}_3$  M/1000, .5 cc.; sucrose M/1, 7.3 cc.; water, 30.1 cc.

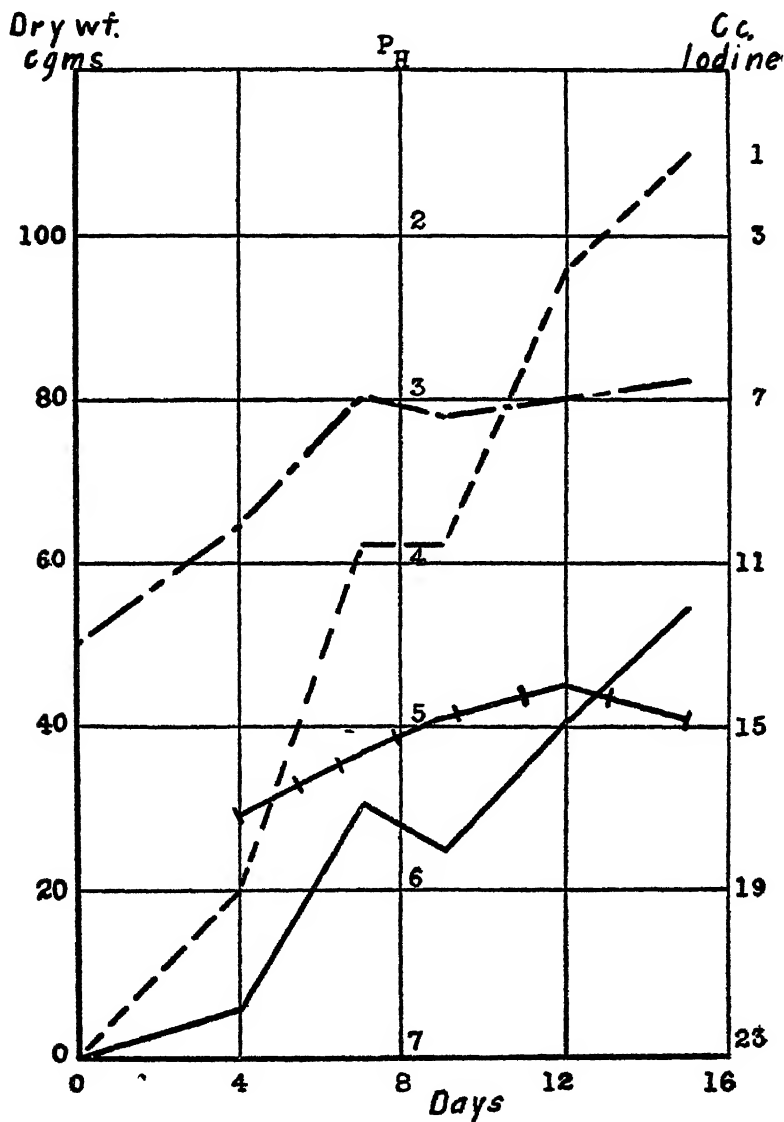


Fig. 9. *Botrytis cinerea* on solution of initial  $P_H$  4.5 containing thio-sulphate.

*Experiment 20.*—The solution used in this experiment, with an initial  $P_H$  4.1, was that given above without the addition of acid or alkali. The growth of the 3 fungi is uniformly better in

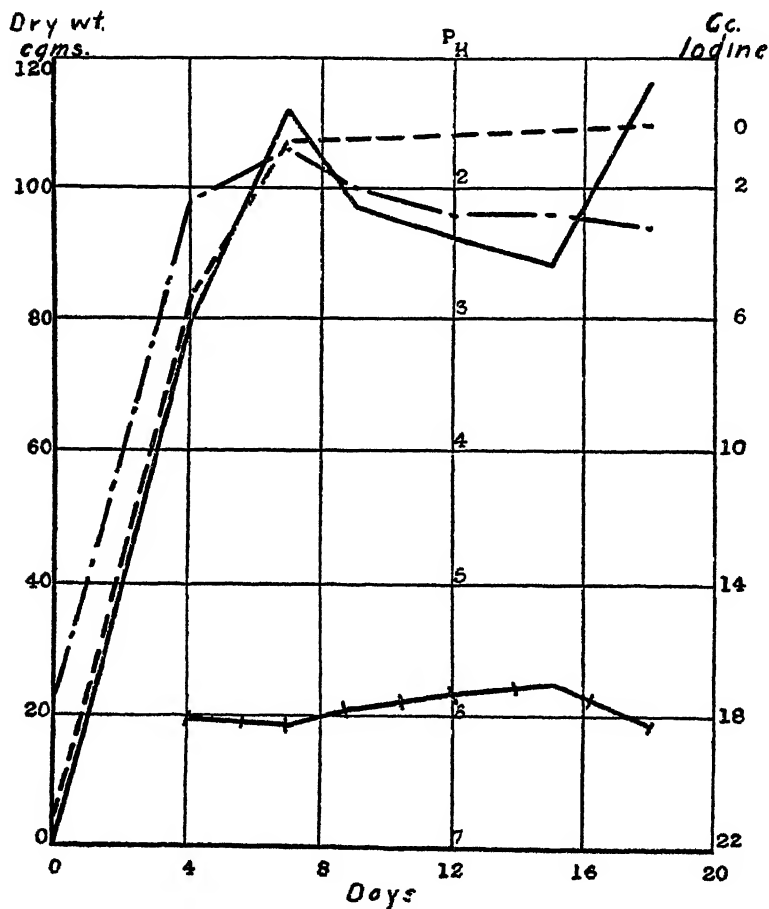


Fig. 10. *Aspergillus niger* on solution of initial  $P_H$  5.9 containing thiosulphate.

these cultures than where the thiosulphate is used at the same  $P_H$ . This is rather markedly so for *Botrytis* which is most susceptible to the inhibiting influence of the thiosulphate. The acidity produced by *Aspergillus* and *Botrytis* is also greater than that in any of the cultures of the 4 preceding experiments.

The tests for sugar were made by adding a few cc. of Fehling's

solution to 10 cc. of the culture solution which was then warmed. If no precipitate appeared, a few drops of HCl were added to another 10-cc. portion of the culture, this brought to a boil, and Fehling's solution added as before. The latter step was not

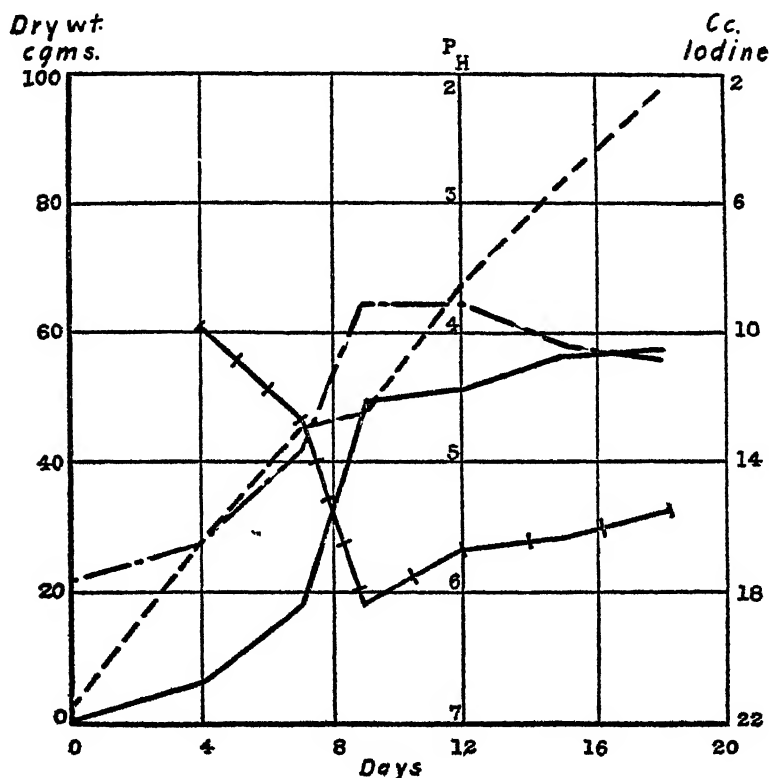


Fig. 11. *Penicillium cyclopium* on solution of initial  $P_H$  5.9 containing thiosulphate.

necessary in any case where sugar was present, for there appeared to be some inversion even in the control flasks. A comparison of the changes in reaction caused by the different fungi can be seen from table xiv and figs. 16, 17, and 18. The peak of growth for *Aspergillus* is reached by the seventh day, with a total disappearance of the sugar and only a slight decrease in the acidity from  $P_H$  1.5 to 1.9. The peak of growth was not reached by *Botrytis* even at the eighteenth day, neither had the sugar been



entirely used, nor was there any reversion of the reaction that might not be due to experimental error. The temperature of these experiments is not as favorable for *Botrytis* as for the other organisms, and no doubt this is one factor in its less rapid growth. The peak of growth was reached for *Penicillium* by the twelfth

TABLE XV

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING MAGNESIUM SULPHATE. INITIAL  $P_H$  5.5. EXPERIMENT 21

| Fungus   | Days | Dry wt.<br>(gms.) | Final<br>$P_H$ | Sugar  | Sulphates | H <sub>2</sub> S | Sporulation |
|----------|------|-------------------|----------------|--------|-----------|------------------|-------------|
| A. nig.  | 4    | .8130             | 1.9            | +      | +         | 0                | 0           |
|          | 7    | 1.0426            | 1.5            | 0      | +         | 0                | 1           |
|          | 10   | .8499             | 2.1            | 0      | +         | 0                | 2           |
|          | 12   | .7549             | 1.9            | 0      | +         | 0                | 2           |
|          | 15   | .7409             | 2.9            | 0      | +         | 0                | 3           |
|          | 18   | .6072             | 2.9            | 0      | +         | 0                | 4           |
| P. cycl. | 4    | .2466             | 5.3            | +      | +         | 0                | 0           |
|          | 7    | .9028             | 4.1            | +      | +         | 0                | 1           |
|          | 10   | 1.1006            | 4.7            | +      | +         | 0                | 1           |
|          | 12   | 1.0497            | 5.0            | Slight | +         | 0                | 1           |
|          | 15   | .8440             | 5.0            | 0      | +         | 0                | 1           |
|          | 18   | .9560             | 5.1            | 0      | +         | 0                | 1           |
| B. cin.  | 4    | .1480             | 5.3            | +      | +         | 0                | 1           |
|          | 7    | .4881             | 4.3            | +      | +         | 0                | 3           |
|          | 10   | .7611             | 3.6            | +      | +         | 0                | 3           |
|          | 12   | .8719             | 2.9            | +      | +         | 0                | 3           |
|          | 15   | .8645             | 2.9            | +      | +         | 0                | 3           |
|          | 18   | .9728             | 3.1            | +      | +         | 0                | 3           |
| Control  |      |                   | 5.5            | +      | +         | 0                |             |

day, with sugar present in small amounts the fifteenth day, though a reversion of the reaction began after the seventh day and proceeded from  $P_H$  3.7 to 5.0, when the sugar had disappeared from the solution.

*Experiment 21.*—To produce a solution with an initial  $P_H$  of 5.5, 5 cc. of sterile NaOH were added to each flask, hence this quantity of water was deducted from that added to each flask in

experiment 20. The different relations of the fungi to the changes produced can be seen in table xv and figs. 19, 20, and 21. In comparing these results with those in experiment 18 where the same amount of sodium hydroxide was added to the thio-

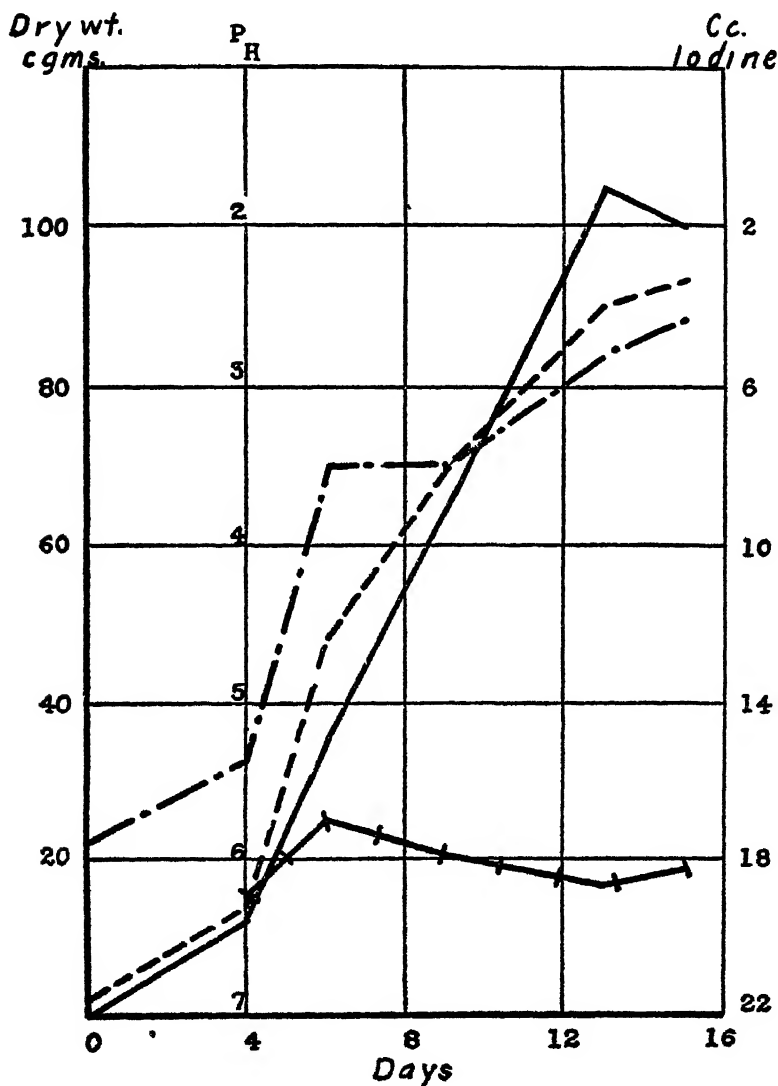


Fig. 12. *Botrytis cinerea* on solution of initial  $P_H$  5.9 containing thiosulphate.

sulphate cultures, however with a slightly different  $P_H$ , it can be seen that the growth of *Aspergillus* is less, that of *Botrytis* about the same, and that of *Penicillium* practically double. Both *Aspergillus* and *Penicillium* exhibit a reversion of the reaction,

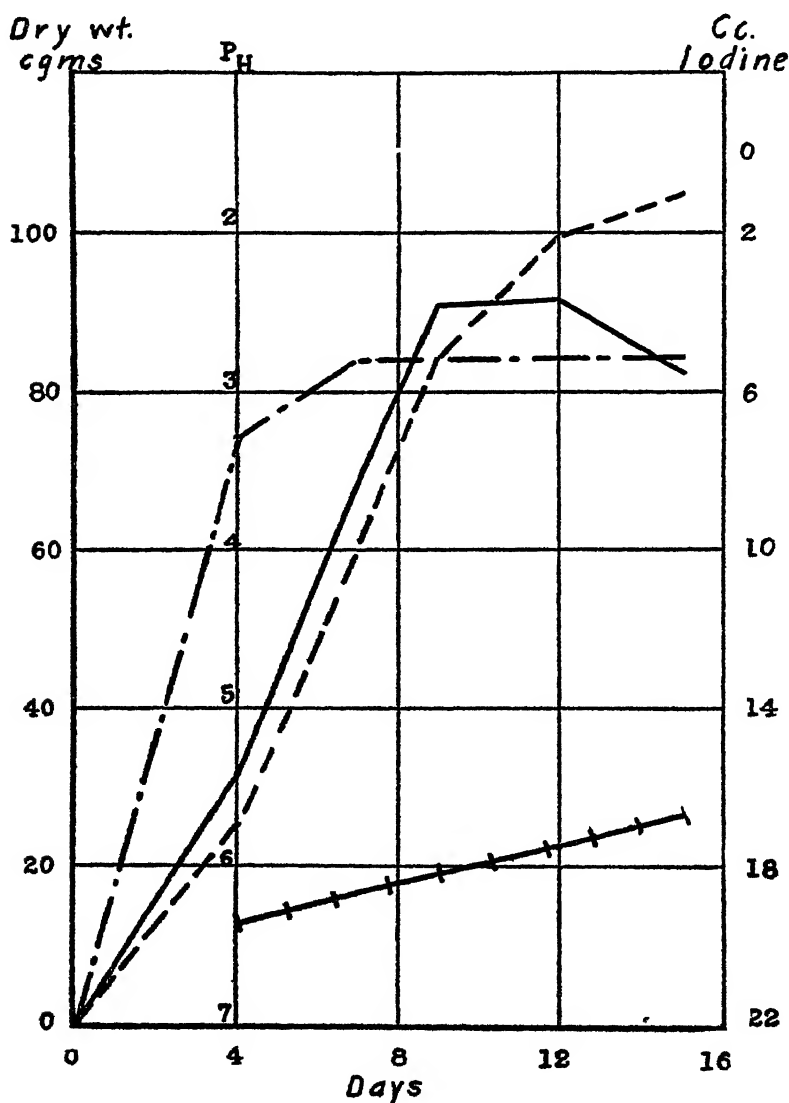


Fig. 13. *Aspergillus niger* on solution of initial  $P_H$  7.1 containing thiosulphate.

the reversion becoming apparent in cultures supporting *Aspergillus* after the seventh day, when sugar was no longer present; while the reversion with *Penicillium* occurred at the same period, though sugar was present until after the twelfth day. The change in reaction for *Aspergillus* was from  $P_H$  1.5 to 2.0 and for *Penicillium* from  $P_H$  4.1 to 5.1.

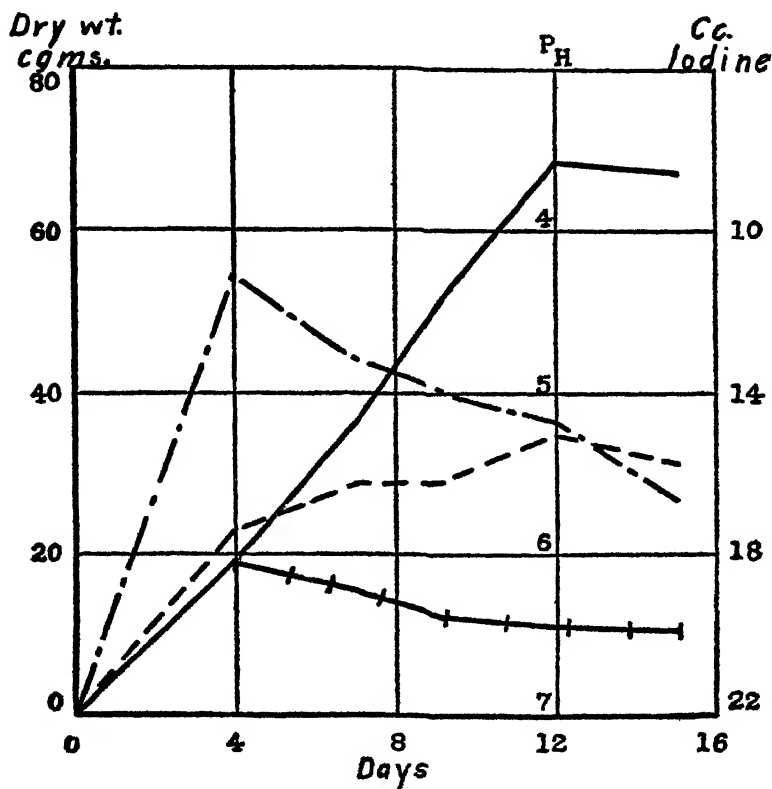


Fig. 14. *Penicillium cyclopium* on solution of initial  $P_H$  7.1 containing thiosulphate.

No  $H_2S$  was produced from any culture where magnesium sulphate served as a source of sulphur.

#### DISCUSSION

In all the cultures with  $Na_2S_2O_3$  as a source of sulphur, sulphates appear as the chief end product of the action of the fungus

on this compound,  $H_2S$  is generally produced, and extracellular sulphur, the tetrathionate, and also globules of sulphur in the hyphae sometimes occur.

Nathansohn ('02), Tanner ('17), Beijerinck ('00, '04), Neuberg

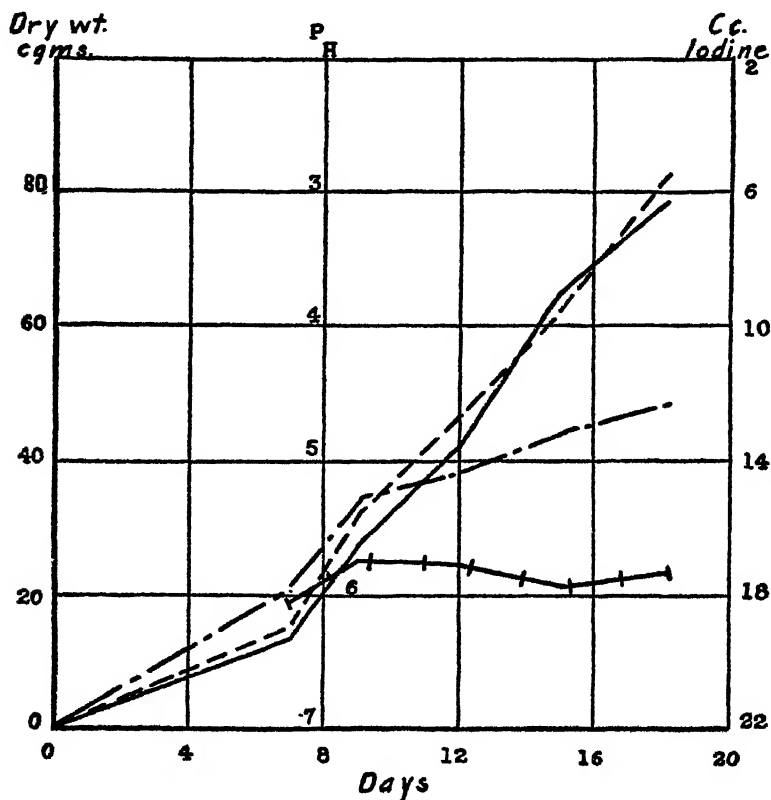


Fig. 15. *Botrytis cinerea* on solution of initial  $P_H$  7.1 containing thiosulphate.

and Welde ('15), and Raciborski ('05), working with bacteria and higher fungi, have presented equations representing the possible course of the changes involved, the variations in the equations depending upon the chief end product. The 3 fungi used have produced all of the above-named compounds in one or the other of the solutions employed, hence it has seemed inadvisable to give a specific equation representing the probable nature of the metabolic changes.



production to the known factors. For example, *Aspergillus* has produced  $H_2S$  from every solution containing thiosulphate except the one with initial  $P_H$  4.5, lowest  $P_H$  1.7, where all the

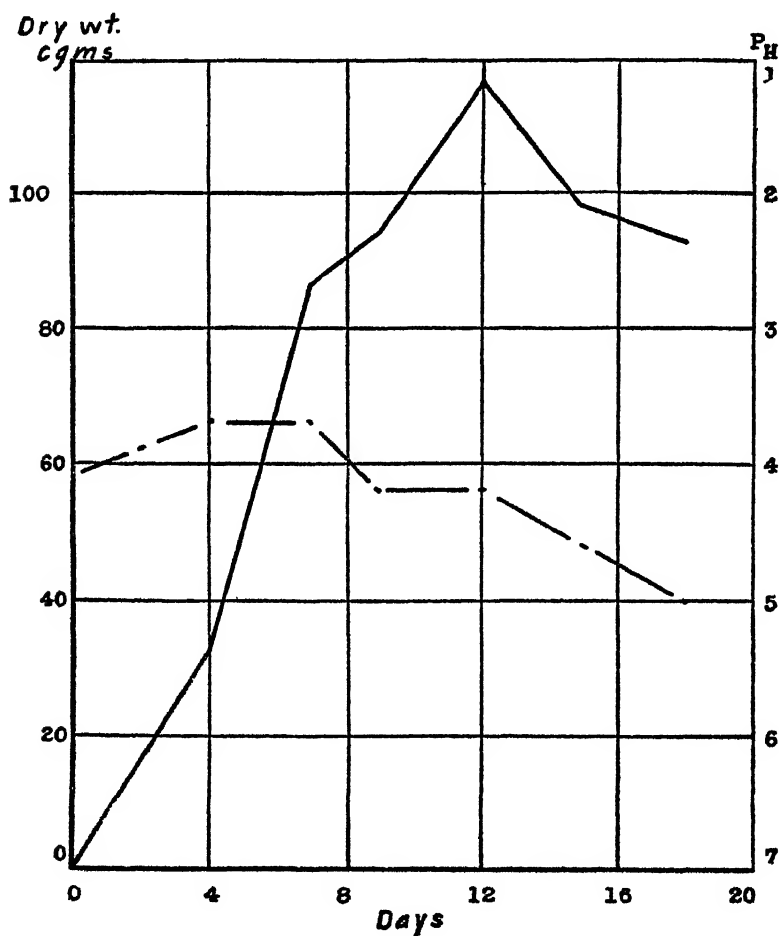


Fig. 17. *Penicillium cyclopium* on solution of initial  $P_H$  4.1 containing magnesium sulphate.

thiosulphate was decomposed and sulphates were clearly apparent. *Penicillium* has behaved similarly except on the solution with the initial  $P_H$  7.1, lowest  $P_H$  4.4, where the growth was good, yet with little of the thiosulphate decomposed. The initial acidity alone might be conceived as the limiting factor, for the

actual acidity during much of the growing period was practically the same in the two cases cited as for other experiments.

A separation of free sulphur has occurred, more particularly at the higher concentrations. That the deposit is sulphur, or

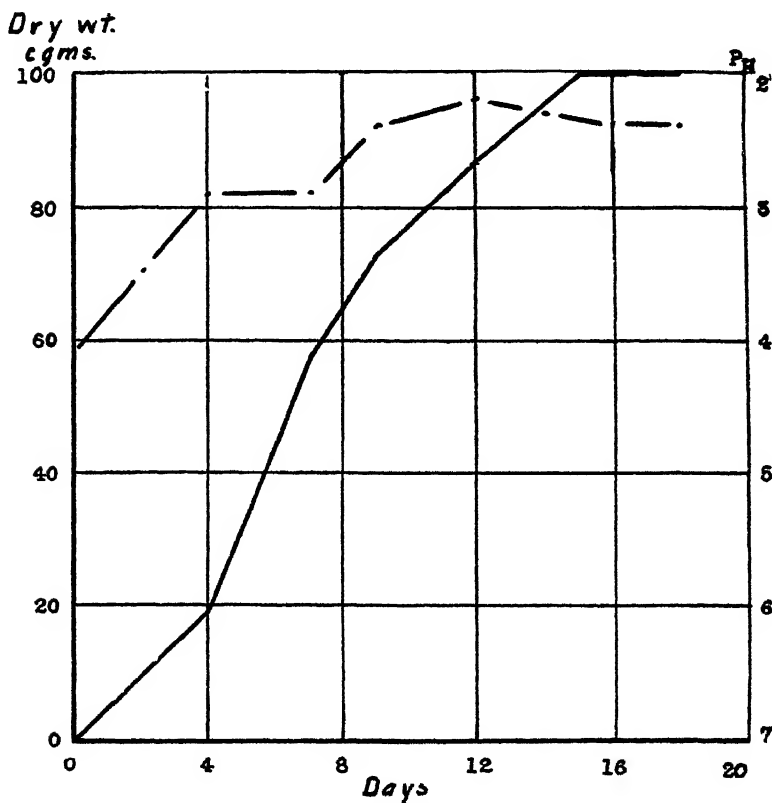


Fig. 18. *Botrytis cinerea* on solution of initial  $P_H$  4.1 containing magnesium sulphate.

largely so, is shown by the easy solubility of most of the deposit in  $CS_2$  and by the distinct odor of sulphur obtained on drying and heating the deposit. Globules like those in milk of sulphur, as well as many rhombic and monoclinic crystals, were found in the cultures with the higher concentrations of the thiosulphate. Nathansohn ascribed the separation of sulphur in his cultures to the production of a tetrathionate, while Raciborski could find no tetrathionate but observed the separation of sulphur. In the



solution employed in experiment 3 in which *Aspergillus* evidently caused the production of a tetrathionate, there was no apparent separation of sulphur, while in several other cultures where the separation occurred no tetrathionate could be detected.

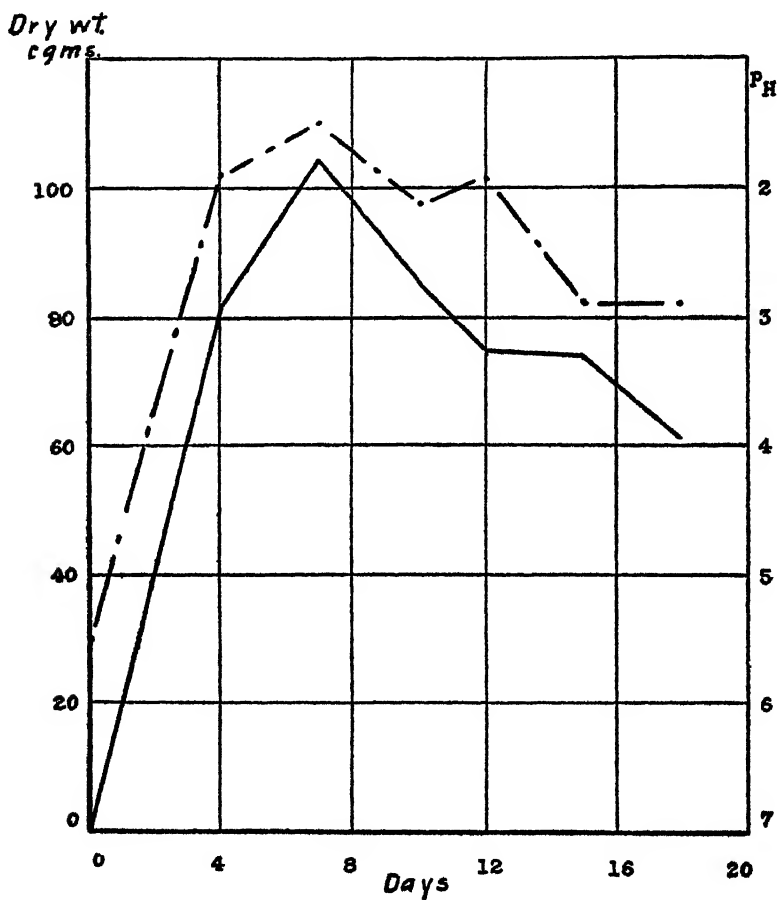


Fig. 19. *Aspergillus niger* on solution of initial  $P_H$  5.5 containing magnesium sulphate.

The tips of many hyphae contained globules of varying sizes after a growth period of 21 days in the 5 and 10 per cent solutions of thiosulphate. A few days after the end of this period, some of the globules were found crystallizing in the hyphae in the shape of double pyramids as described by Molisch ('13) and Miyoshi

('97). These globules and crystals give the reactions and have the appearance of sulphur, and they show that these fungi can, under certain conditions, accumulate sulphur in the mycelium as

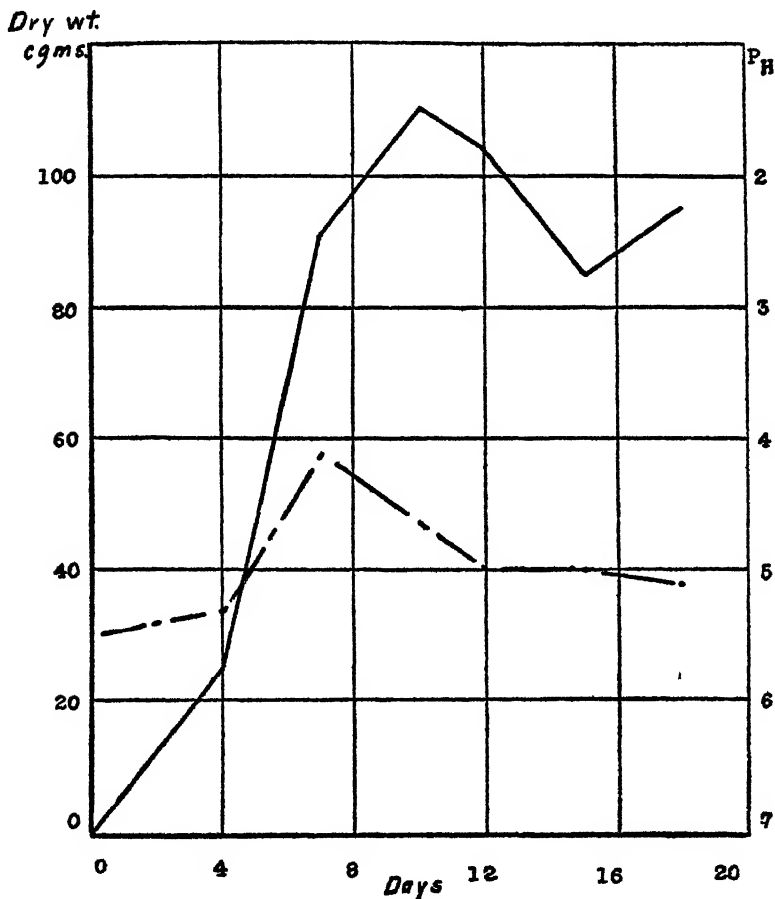


Fig. 20. *Penicillium cyclopium* on solution of initial  $P_H$  5.5 containing magnesium sulphate.

in the case of the filamentous sulphur bacteria. The solubility of the globules in carbon bisulphide, ether, and chloroform, and their insolubility in hydrochloric acid, absolute alcohol, hot alkali (KOH), glacial acetic acid, nitric acid, benzol, and benzol after treatment with absolute alcohol, indicate that they are sulphur.

In experiments 16-19 inclusive, in which the thiosulphate was used as a source of sulphur, the ratio of thiosulphate decomposed to growth is not a constant in all cases, but the constant relation does appear in 8 of 12 sets of cultures as shown in figs. 4, 5, and 6. The hydrogen-ion concentration of the medium does not appear to be a limiting factor in the efficiency of the use of the thiosulphate.

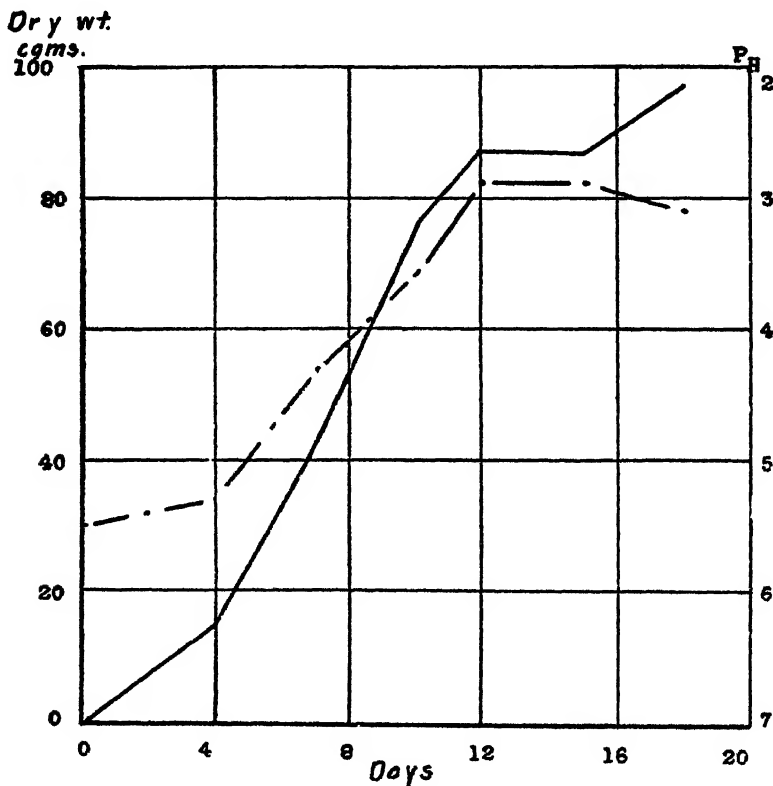


Fig. 21. *Botrytis cinerea* on solution of initial  $P_H$  5.5 containing magnesium sulphate.

Reversions of the reaction from the more acid condition towards neutrality were observed with both *Aspergillus* and *Penicillium*. From the results of experiments 20 and 21 it is seen that *Aspergillus* caused a rapid decomposition of the sugar, that the total disappearance of the sugar is accompanied by the production

of high acidity, and that a reversion of the reaction immediately follows. *Penicillium cyclopium* caused the reversion to take place before the disappearance of the sugar, suggesting the simultaneous production of hydrogen and hydroxyl ions with the hydroxyl ions produced in excess. Chambers ('20) has studied some interesting relations of the effect of the concentration of the sugar upon the reversion of the reaction by *Bacillus coli* and *Bacillus aerogenes*. Ayers and Rupp ('18) have explained the simultaneous production of acid and alkali by *B. aerogenes* in an inorganic medium as due to the production of organic acids from the sugar with the subsequent formation of alkaline carbonates or bicarbonates from the organic acids.

The results of the determinations of the hydrogen-ion concentration of the medium at relatively short intervals make it apparent that the method of determining the initial and final hydrogen-ion concentrations of fungous cultures may not give an indication of the changes which have proceeded in the reaction.

Sporulation was retarded or largely inhibited in the more acid solutions. *Aspergillus niger* produced the greatest acidity and sporulated at a higher acidity (e. g.,  $P_H$  1.7–2.1) than either *Penicillium* or *Botrytis*. The heavy sporulation of *Penicillium cyclopium* in the solution with an initial  $P_H$  4.2 occurred during the rapid reversion of the reaction when the acidity of the solution was  $P_H$  3.0 or above.

### CONCLUSIONS

1.  $MgSO_4$ ,  $Na_2S_2O_3$ ,  $MnSO_4$ , KSH,  $KHSO_3$ ,  $K_2S_2O_8$ , KCNS, and  $NH_4CNS$ , in general, have served as favorable sources of sulphur, in the order named, for *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea*. Meagre growth was obtained with  $K_2S$ . Inhibition of growth occurred for *Penicillium* on  $K_2S_2O_8$  though this compound was better for *Aspergillus*, in the concentration employed, than KSH or  $KHSO_3$ .

2.  $H_2S$  has been produced except where  $MnSO_4$ ,  $MgSO_4$ , and  $K_2S_2O_8$  were used. The production of this compound seems unrelated directly to hydrogen-ion concentration, concentration of the salt, or relative degree of growth.

3. If the culture solution, sulphates appear as the chief end product of the action of the above-named fungi on  $Na_2S_2O_3$ ,

H<sub>2</sub>S is generally produced, molecular sulphur in visible quantity not infrequently appears, the tetrathionate has been identified in certain cases, and in the hyphae globules of sulphur sometimes occur.

4. The ratio of thiosulphate decomposition to growth is not a constant in all cases for *Aspergillus niger*, *Penicillium cyclopium*, and *Botrytis cinerea*, though in the 12 series of cultures here reported upon such a constant relation does appear with one or more of the fungi in 8 of the series. The usual growth range of hydrogen-ion concentration does not appear to be a limiting factor in the efficiency of the thiosulphate as a source of sulphur for these fungi.

5. In a modified Pfeffer's solution the disappearance of the sugar, within the limits determined, marks the point of the reversion of reaction for *Aspergillus niger*. *Penicillium cyclopium*, on the other hand, may cause a reversion of the reaction with sugar present in the solution.

6. Since it has been established that reversion of the reaction may occur, it is clear that the true course of the changes which have occurred may not be obtained merely by a determination of the initial and final hydrogen-ion concentrations of the fungous cultures.

The writer wishes to express his appreciation of the invaluable suggestions and criticisms of Dr. B. M. Duggar in the later investigations which are the subject of much of this paper. Thanks are extended to Dr. J. B. Overton, of the Department of Botany of the University of Wisconsin, for helpful direction and advice in the early progress of this work. Thanks are also due Dr. George T. Moore for the privileges and facilities of the Missouri Botanical Garden.

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# STUDIES IN THE PHYSIOLOGY OF THE FUNGI

## XV. GERMINATION OF THE SPORES OF CERTAIN FUNGI IN RELATION TO HYDROGEN-ION CONCENTRATION<sup>1</sup>

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### INTRODUCTION

The effect of the hydrogen- and the hydroxyl-ion concentration in a mannite medium upon germination of the spores of certain fungi, *Aspergillus niger*, *Penicillium cyclopium*, *Botrytis cinerea*, *Fusarium* sp., and *Lenzites saepiaria*, and the range within which their most favorable germination occurs have been presented in a recent paper (Webb, '19). Very striking data were obtained from this preliminary study, the features of particular interest being (1) the importance of active acidity in germination and (2) the relatively low percentage of germination under conditions of active alkalinity. It was found, in general, that the majority of the fungi employed showed maximum germination of the spores with relatively high acidity, namely,  $P_H$  3.1, and further, that none of the fungi suffered inhibition of germination on the acid side of neutrality until a hydrogen-ion concentration greater than that of  $P_H$  2.8 was passed. Germination quantities in most cases decreased, though not necessarily proportionally, with decrease in hydrogen-ion concentration from the optimum concentration previously mentioned. Certain forms, however, such as *Penicillium cyclopium* and *Fusarium* sp., exhibited secondary maxima at or near neutrality.

The medium employed in that investigation contained mannite as the sole nutrient, and its hydrogen- and hydroxyl-ion concentrations were adjusted by means of equal additions of orthophosphoric acid and successively increasing additions of sodium hydroxide. Logical questions which naturally arise at this

<sup>1</sup> An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

point are: Is the effect of the hydrogen- and hydroxyl-ion concentration upon the rate of germination of the spores of certain fungi the same in different types of media? Is the range within which the most favorable germination occurs the same for different types of media? What relation does nutrition bear, if any, to the toxicity of the hydrogen- and hydroxyl-ion concentration during germination? And, if certain differences in effect exist, what are the explanations of such phenomena? These questions, together with others, suggested the desirability of conducting the investigation reported in this paper. Using the spores of 8 fungi a comparative study has been made dealing with the effects of hydrogen- and hydroxyl-ion concentrations upon germination (1) in water, (2) in such single nutrient solutions as mannite and peptone, and (3) in such full nutrient solutions as Czapek's solution and sugar-beet decoction.

#### LITERATURE

The literature concerning the germination of fungous spores and their subsequent growth and development, as related to the reaction of the medium, has been historically considered by the writer in an earlier paper (Webb, '19). These articles together with certain others—as reviewed below—are completely cited in the bibliography of this paper.

Buller ('06) obtained a high germination percentage with the spores of *Polyporus squamosus* in a malt-wort extract, the reaction of which was slightly acid. He prepared a solution with tap water, meat extract, 0.5 per cent, peptone, 0.5 per cent, grape sugar, 3 per cent, and gelatin, 10 per cent, adding sodium carbonate until distinctly alkaline. The mycelium grew in this mixture more vigorously and branched more frequently than in the malt-wort extract. In a decoction made from the wood of *Acer pseudoplatanus*, no germination occurred during the first 2 days, but about 1 per cent of the spores had germinated on the third day. However, bacteria had developed abundantly in the culture. Buller says: "The impression given me by this exceptional case was that the metabolism of the bacteria had given rise to some substance which to a slight extent had stimulated the spores to germinate." A change in reaction of the medium may have been a contributing factor, but no mention is made concerning this point.

Falck ('12) showed that the acidity of the medium is a conditioning factor for the growth of several species of *Merulius*. The same writer further observed that *Coniophora*, by making the medium decidedly acid and thus providing favorable conditions for the germination of the spores, conduced to the subsequent growth and development of *Merulius*.

Peltier ('12) obtained best development of *Botrytis cinerea* on various culture media possessing a slightly acid reaction and poorest growth on strongly alkaline media. A strongly alkaline medium, moreover, caused the mycelium to remain sterile, while a strongly acid one reduced the number of sclerotia and favored conidial production.

Stakman ('13), in an extensive study on the germination of cereal smuts, found that the period required for germination and the morphological features during germination varied with the organism. In general, greater vigor of germination and more abundant and prolonged production of sporidia occurred in sugar solutions than in water.

According to Cooley ('14), *Sclerotinia cinerea* may even grow on a medium as acid as the natural juice of sour plums or cherries, although it develops more luxuriantly on a somewhat less acid medium. No perceptible growth was immediately produced with a neutral reaction of the medium, but at the expiration of 2 weeks the mycelial growth at such a reaction nearly equaled that on the acid medium. Spore production was very abundant on the acid media, but entirely inhibited on the alkaline side. While the fungus required relatively high acidity for maximum growth, it could adjust itself in time to a slight degree of alkalinity.

Gillespie ('18) studied the growth of certain strains of *Actinomyces chromogenus* as related to various hydrogen-ion concentrations within the range  $P_H$  4.8-7.2, using succinate, citrate, and potato-tartrate media. In general, the potato-scab organism was found to be inhibited in culture media with an acidity of  $P_H$  5.2, and better growth developed at less acid reactions. Individual strains showed different sensitiveness to acidity, but the differences failed to furnish any consistent distinctions. Growth in the most acid cultures was accompanied by a marked decrease of acidity in the medium, but such changes varied with the organism, the medium, and the initial reaction.

Steinberg ('19), using Pfeffer's solution as a medium, conducted experiments dealing with the relation of 7 different acids to the growth and spore production of *Aspergillus niger*. The addition of acid to the normal nutrient solution, which initially tests  $P_H$  3.0-4.0, produced an acceleration of growth; the addition of alkali to the normal nutrient solution resulted in a diminution in yield. The effect of the increased acidities of the cultures was to cause a retardation or suppression of spore formation as contrasted with the effect of decreased acidity, which failed to exhibit the same phenomenon. All 7 acids gave similar results, indicating that the results are primarily due to the hydrogen ions rather than to the anions.

Zeller, Schmitz, and Duggar ('19), growing various wood-destroying fungi on Czapek's solution, Dunham's solution, a pine decoction, Reed's solution, Richards' solution, and sap from *Acer saccharinum*, whose reactions were adjusted by means of mono-, di-, and tri-basic potassium phosphate, found that there is a decided indication of the advisability of selecting a specific medium for each fungus. The hydrogen-ion concentration did not seem to be the only limiting factor in growth, and the shifting of the hydrogen-ion concentration due to metabolism depended both upon the medium and the fungus. No general statement, in their opinion, could be made concerning the relation between hydrogen-ion concentration of the culture media and the growth of wood-destroying fungi as a group.

Thiel and Weiss ('20) report that acetic acid in aqueous solution exerts a pronounced stimulating effect on the germination of teliospores of *Puccinia graminis*. Other acids and chemical agents in various concentrations were employed by them, but all gave negative results. Although no hydrogen-ion determinations were made, or at least published, the authors are inclined to believe that the process is not one of hydrogen-ion catalysis, but one of a specific activator.

Zeller ('20) obtained a high percentage of spore germination of *Lenzites saepiaria* upon the shavings of short-leaf pine sapwood when the humidity of the air was sufficiently high to supply free water as a film on the wood surface. A decoction prepared by steaming such shavings with distilled water in a reflux tested

$P_H$  4.2, with active acidity of the shavings probably not above  $P_H$  3.8. This closely approaches  $P_H$  3.0, the optimum hydrogen-ion concentration in various media, as reported in this paper by the writer.

Hopkins ('21), varying the hydrogen-ion concentration of a synthetic medium, the name of which does not appear in the published abstract, by means of  $KH_2PO_4$ ,  $K_2HPO_4$ ,  $H_3PO_4$ ,  $H_2SO_4$ , KOH, and NaOH, found that the amount of growth of *Gibberella Saubinetii* increased with decreasing acidity from  $P_H$  2.5 to a maximum at  $P_H$  4.0–4.5. It then decreased to a minimum at  $P_H$  5.0–5.5 and rose again to a second maximum, but the highest point was not determined. Conidial germination of the same organism also showed a double maximum. A *Fusarium* isolated from scabby wheat, but not proved to be *Gibberella*, exhibited a similar depression in the growth-acidity curve. These relations agree well with those established in this paper.

Armstrong ('21), using a modified Richards' solution containing sulphur supplied as  $Na_2S_2O_3$  and as  $MgSO_4$ , determined the growth relations of certain fungi as influenced by hydrogen-ion concentration. Within the experimental range  $P_H$  3.0–7.1, he found that *Aspergillus niger* produced maximum growth with an initial reaction of  $P_H$  5.5–5.9, *Penicillium cyclopium* at  $P_H$  4.1–4.5, and *Botrytis cinerea* at  $P_H$  4.1–5.9. Inhibition of growth, in all cases, occurred at  $P_H$  3.0, and in the case of *Botrytis cinerea*, distinctly retarded and suppressed growth was evidenced at  $P_H$  7.1. Changes in reaction of the liquid culture media invariably took place during growth of the organisms, and, in addition, reversions in the reaction frequently manifested themselves, the nature and degree of such changes and reversions varying with the organism, the medium, and the initial hydrogen-ion concentration.

Karrer ('21), growing certain fungi on Czapek's solution with soluble starch as the source of energy, accumulated interesting data with respect to the reaction of the medium. *Fusarium* produced no growth at  $P_H$  2.0 and the limit on the alkaline side extended beyond  $P_H$  9.2. *Colletotrichum Gossypii* gave a fair quantity of mycelium from between  $P_H$  3.0–4.5 to above  $P_H$  9.2. *Penicillium italicum* displayed a more limited range for favorable

growth. Best results were obtained from  $P_H$  2.5 to  $P_H$  4.5, and only a few hyphae were produced at  $P_H$  8.0. During the growth of the organisms, certain shifts in the reaction of the medium were evidenced. In the initially acid cultures, *Fusarium* and *Colletotrichum* produced a shift towards alkalinity, while the reaction of the initially alkaline cultures remained more or less stable; in the case of *Penicillium* the initially acid cultures tended to shift towards neutrality.

#### MATERIALS AND TECHNIQUE ORGANISMS

Regarding the fungi and the media employed, the original plan of study was designed primarily to be one of a general nature. Fungi possessing widely divergent cultural relations with respect to the reaction of the medium, that is, fungi producing maximum growth at markedly different hydrogen-ion concentrations, would naturally furnish, it was realized, opportunity for the most interesting data; hence these were given chief consideration. In addition, it seemed desirable to select such economically important forms as might suitably serve to represent certain significant fungous groups. Accordingly, although only relatively few fungi were obtained which would germinate freely in water, the following were chosen and, from previous knowledge of their behavior, differentiated into provisional groups as follows: Acid forms—*Botrytis cinerea*, *Aspergillus niger*, *Penicillium cyclopium*, *P. italicum*, *Puccinia graminis*, and *Lenzites saepiaria*; acid and alkaline forms—*Fusarium* sp.; alkaline forms—*Colletotrichum Gossypii*. The fungi mentioned were obtained from various sources: *B. cinerea*, isolated from lettuce plants growing in the greenhouse; *A. niger* and *P. cyclopium* from a jar of beans which had become contaminated in the laboratory; *P. italicum*, from oranges grown in California; *P. graminis*, a strain which was obtained from Berkeley, California; *L. saepiaria*, from sporophores collected at intervals from railroad ties in the vicinity of St. Louis; *Fusarium* sp., isolated from a cotton boll; and *C. Gossypii*, furnished by the South Carolina Agricultural Experiment Station.

In the test-tube cultures from which the spores were taken, *B. cinerea*, *A. niger*, *P. cyclopium*, and *Fusarium* sp. were grown on potato agar made according to Duggar, Severy, and Schmitz ('17); i. e., 230 gms. of potato were cut into small pieces, autoclaved in 1 liter of water for 1 hour at 15 pounds pressure, and filtered while hot. Fifteen gms. of agar were then added, and the mixture was autoclaved for 15 minutes at 15 pounds pressure, and, correction having been made for loss of water, was finally tubed, sterilized, and slanted. *Penicillium italicum* frequently produced spores with difficulty, and the limiting factor seemed to be the reaction of the medium. A medium of relatively high acidity is essential for sporulation with this organism, and the medium here employed was 1.5 per cent agar made up in Czapeks' full nutrient solution as outlined on page 290. *Colletotrichum Gossypii* was grown on agar conforming to a formula suggested by Professor Barre: peptone, 10.0 gms.; glucose, 15.0 gms.;  $MgSO_4$ , 0.25 gm.;  $K_2HPO_4$ , 0.25 gm.; agar, 15.0 gms.; and  $H_2O$ , 1000 cc. Cultures grown in the light produced abundant spores; those grown in subdued light, relatively few; and those in the dark, none. All cultures were allowed to grow at room temperature, and the spores were always taken from cultures ranging in age from 10 to 15 days.

In the case of *P. graminis*, wheat seedlings were inoculated with uredospores and allowed to develop within a cheese-cloth cage. Spores to be used for germination were always taken from fresh sori, usually appearing from 10 to 18 days after inoculation. Spores of *L. saepiaria* were obtained according to the pure culture method outlined by Zeller ('16). The sporophores were first rinsed several times in sterile distilled water in large test-tubes and then allowed to stand for about an hour in sterile distilled water. Following this they were removed with sterile forceps, the surplus water being removed by drying with sterile tissue toweling, and were finally placed, hymenium downward, in large, dry, sterile Petri dishes. After 24-48 hours the sporophores had discharged sufficient spores to make a white spore print.



## CULTURE SOLUTIONS

*Mannite*.—The titration curves of the various liquid media employed in this study are shown in fig. 1. The composition of the single nutrient or mannite culture solutions was based primarily upon the Clark and Lubs ('17) titration curve of orthophosphoric acid, and the methods of procedure are the same as those previously described by the writer ('19). Stock solutions of M/5 mannite in M/10  $H_3PO_4$  and M/5 mannite in N/5 NaOH were made. Equal quantities of the M/5 mannite-M/10  $H_3PO_4$  solution were placed in each Pyrex flask, and successively increasing proportions of M/5 mannite-N/5 NaOH were added. The flasks were plugged with cotton, sterilized at 15 pounds pressure for 15 minutes, and allowed to stand at least 24 hours before making H-ion determinations. Perfect agreement between calculated and determined values always resulted except within the range  $P_H$  8.0–10.0 where a shift towards neutrality frequently occurred. Such deviations, however, might well be attributed to the extreme lack of buffer action within this range, as shown by fig. 1, and also to any hydrolysis of mannite that might occur during sterilization. A relatively small precipitate invariably occurred in the extreme alkaline cultures.

*Czapek's solution*.—Czapek's full nutrient solution was made according to the formula published in a recent paper by Zeller, Schmitz, and Duggar ('19):  $MgSO_4 \cdot 7H_2O$ , 0.5 gm.;  $KH_2PO_4$ , 1.0 gm.; KCl, 0.5 gm.;  $NaNO_3$ , 2.0 gms.;  $FeSO_4$ , 0.01 gm.; cane sugar, 30.0 gms.;  $H_2O$ , 1000 cc. The original nutrient solution was prepared by adding 600 cc., instead of 1000 cc., of distilled water. This method, which has been described by Karrer and Webb ('20), and which will be restated briefly, allows dilutions of the various solutions by the addition of regulated amounts of acid or alkali and water for the adjustment of various H-ion concentrations without materially affecting the concentrations of the nutrient salts or constituents. The acid and alkali used with this particular medium were N/5 HCl and N/20 KOH. This strength of alkali is more satisfactory than one more concentrated, the latter introducing difficulties in securing those reactions less acid than  $P_H$  4.5–5.2—the H-ion concentration

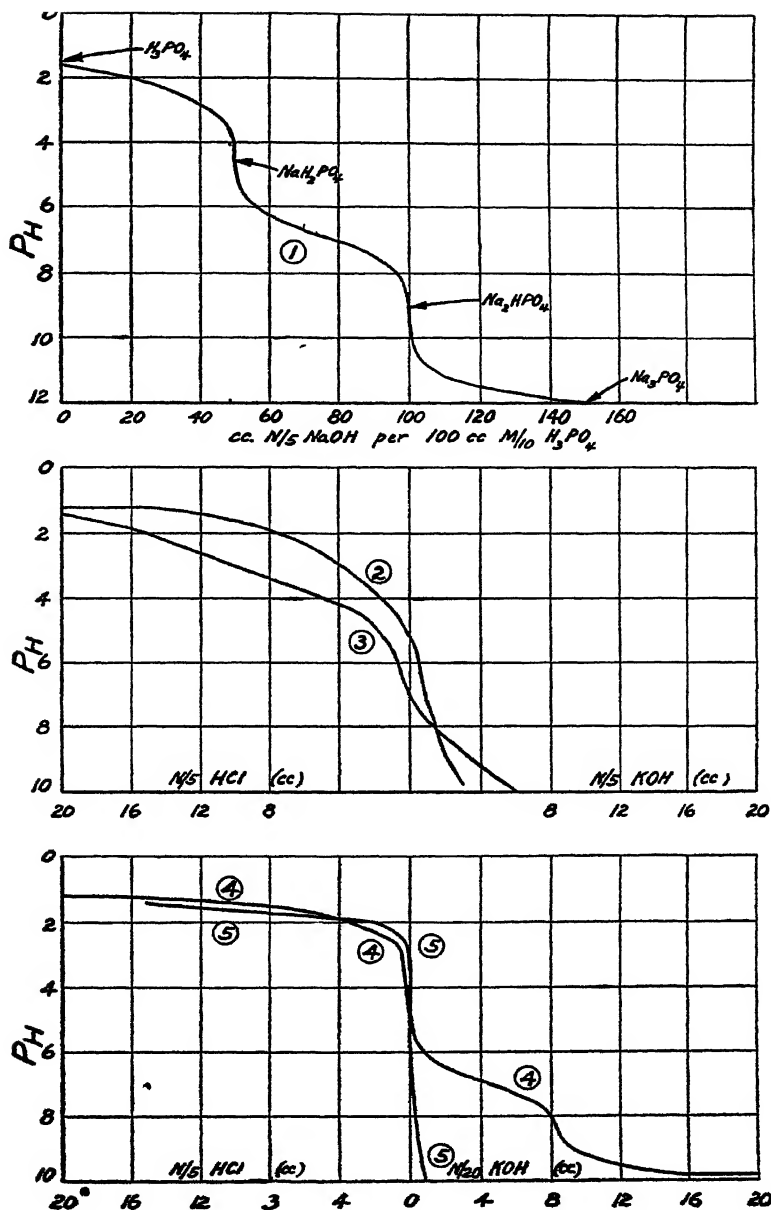


Fig. 1. Titration curves of certain liquid media: (1) orthophosphoric acid (after Clark and Lubs); (2) sugar beet decoction; (3) 2 per cent bacto-peptone solution; (4) Czapek's full nutrient solution (2, 3, and 4 after Karrer and Webb); (5) "water HCl or KOH."

of normal Czapek's solution. With the peptone and the beet decoction solutions, however, N/5 KOH has been used. In all of the media, namely, Czapek, peptone, beet decoction, and water (adjusted by HCl), N/5 HCl was favorable for varying the reactions on the acid side.

Inasmuch as sugars generally react with acid and alkali when heated under pressure, the nutrient solutions and the acid and the alkali were sterilized separately. Thirty cc. of the nutrient solution together with the desired amount of water, the amount of which equals the difference between the total volume and the sum of the volumes of the nutrient solution and the acid or alkali to be added, as shown by fig. 1, were put into small flasks plugged with cotton, and sterilized. After cooling, the cultures were removed to a culture room, and the definite amounts of sterile acid and alkali were added with sterile graduated pipettes. The final volume of each culture was 50 cc., and each case represented a dilution of the constituents comparable with that in the original nutrient solution. The solutions were allowed to stand at least 24 hours in order to reach a state of equilibrium before H-ion determinations were made. A certain small amount of precipitate occurred upon the addition of alkali, the amount increasing with increase of added alkali. Mannite was substituted for cane sugar, called for in the formula, in order that the same carbohydrate might be present in both the single and the full nutrient solutions, the plan being to make the experiments with the 2 types of media as comparable as possible.

*Peptone.*—A 2 per cent bacto-peptone solution, prepared according to the titration curve of Karrer and Webb ('20), was used in this investigation. The 2 per cent bacto-peptone solution normally tests  $P_H$  7.0, and the hydrogen-ion concentration was varied with N/5 HCl and N/5 KOH. The buffer action, it will be noticed, is relatively slight on the alkaline side, and is even less apparent in the range  $P_H$  5.0–7.0, the addition of a few tenths of a cubic centimeter of acid producing an almost vertical ascent in the titration curve. With increasing acidity above  $P_H$  5.0, however, buffer action is relatively strong. No precipitation was evidenced in the alkaline cultures of this medium.

*Beet Decoction.*—The sugar beet decoction was prepared according to the method outlined by Duggar, Severy, and Schmitz ('17). This consists essentially of 370.4 gms. of sugar beets per liter of distilled water, autoclaved at 15 pounds for 1 hour and then filtered. Karrer and Webb's ('20) titration curve and method of varying the hydrogen-ion concentration have been used as a basis, and the detailed remarks for Czapek's full nutrient solution adequately suffice in this case. The beet decoction was made in concentrated form and in sufficient quantity for all of the experiments here reported during the late summer of 1920. This decoction, with proper dilution and without addition of either acid or alkali, tested  $P_H$  5.2, and the H-ion concentration was varied on either side by regulated amounts of N/5 HCl and N/5 KOH. The buffer action on the alkaline side is extremely weak, in fact, much weaker than that of bacto-peptone. Without doubt N/20 alkali would have been better than N/5 for the adjustment of reactions in this medium. The stock solutions of beet decoction, however, were made before this point was determined, and it seemed unwise to change the plan at this stage. No noticeable precipitate immediately occurred in the alkaline cultures, although precipitation did occur upon prolonged standing, and a decided color change from pale yellow to amber was noted as the reaction passed from acid to alkaline.

*Water.*—Distilled water was adjusted to different hydrogen-ion concentrations by two methods: (1) by successively increasing additions of N/5 NaOH to equal quantities of M/10  $H_3PO_4$ , (2) by additions of acid or alkali to distilled water. The adjustments in the first case were arranged on the principle described for the mannite culture solutions and the term "water  $H_3PO_4$  and NaOH" has been applied to water so regulated. In the other case distilled water was brought to the required  $P_H$  value by means of N/5 HCl or N/20 KOH and the term "water HCl or KOH" has been employed to designate these solutions. Distilled water testing  $P_H$  5.2–5.4 was the best that could be obtained. The titration curve, as presented, was worked out very hastily and no special precautions have been taken. Despite the fact that it may be subject to some criticism, it is included

for completeness. Initial fluctuations in H-ion concentration generally occurred in the neutral and alkaline cultures.

*Mannite and Beet Decoction.*—In a few experiments, a medium composed of a mixture of mannite and of beet decoction—the two in different proportions in different series—has been used. The method of preparation is a combination of those used with mannite and with beet decoction. First, a series with mannite and one with beet decoction were made. Then equal volumes of the mannite solutions of varying  $P_H$  were placed in flasks and to each was added twice the volume of beet decoction with similar or closely agreeing  $P_H$ . In the other series one volume of beet decoction was first added, and then twice the volume of mannite with the corresponding H-ion concentration. The resulting media were accordingly (1)  $33\frac{1}{3}$  per cent mannite plus  $66\frac{2}{3}$  per cent beet decoction and (2)  $66\frac{2}{3}$  per cent mannite plus  $33\frac{1}{3}$  per cent beet decoction.

Hydrogen-ion concentration determinations of all colorless solutions were made at room temperature according to the usual colorimetric method of Clark and Lubs ('17). The solutions to be tested were, in all cases, allowed to stand at least 24 hours at room temperature in order to establish an equilibrium. Owing to the presence of color in the peptone and in the beet decoction solutions, it was necessary to use a colorimeter for the H-ion determinations. Two types of instruments, a Duboscq (micro) and a Kober have been used at various times in this work, the detailed method of which has been described by Duggar ('19). The H-ion values appearing in the charts, curves, and manuscript, unless otherwise specified, represent initial determinations and not final determinations. Certain changes in reaction during germination occur, but this phase of the topic will be considered subsequently in the discussion.

Solutions ranging in H-ion concentration from  $P_H$  1.2 to  $P_H$  9.2–10.0+ were thus obtained with each medium. Experimental values were, in most cases, identical with the calculated ones, and where the H-ion concentration was beyond the range of the extreme indicator, the reaction has been designated by adding “+” to the last value denoted by the indicator. With any medium the 12 or 13 solutions of varying H-ion concentrations

constituted a series. Certain definite  $P_H$  values regarded as important for this study, as well as for other physiological studies of the fungi, were desired for each medium, for instance,  $P_H$  3.0, the optimum concentration for germination of certain forms in mannite, as shown by previous studies; several concentrations greater than  $P_H$  3.0, in order to determine inhibiting concentrations;  $P_H$  6.5 for the influence of slight acidity;  $P_H$  7.0 for neutrality; and  $P_H$  7.5 for slight alkalinity. Aside from these desired concentrations the range  $P_H$  1.2–10.0+ has been conveniently but not equally subdivided.

Similar technique to that described in my previous paper (Webb, '19) was used throughout this study. For any organism, and usually for several organisms, a stock solution of a specific medium was made up at one time, so that all data in this paper represent uniformity for an organism and a particular medium.

The initial  $P_H$  of any stock solution varied somewhat, variations of several tenths not being infrequent despite the most careful technique during the preparation and the use of highest purity chemicals. Since the highest purity monobasic potassium phosphate then obtainable exhibited a high acidity, the salt was recrystallized until the Sørensen coefficient of  $P_H$  4.529 for 1/15 molecular was obtained.

Doubly distilled water testing  $P_H$  5.2–5.4, distilled first from a Bourdillon still with a block tin condenser, and then redistilled in Pyrex flasks containing several crystals of  $KMNO_4$  and condensed in the usual glass condenser, has been used in all of the experiments here reported.

#### METHOD OF CULTURE

The methods employed, essentially those described by Clark ('99) and Duggar ('01), are substantially those used in my previous work (Webb, '19) and need not be described again. All cultures were run in duplicate and a series with every organism was incubated at three different temperatures, a range of 4–5° C. on either side of an approximate optimum temperature being sufficient for all purposes.

The spores of *C. Gossypii*, it was found, germinate with difficulty in hanging-drop cultures, but germinate more readily in

thin films placed on slides. The slides bearing the spore suspension were elevated upon corks in large Petri dishes, and relatively large quantities of the solution to be tested were placed in the bottom of the dishes. The cultures being destroyed upon examination, it was possible to make only one reading.

In testing the change in reaction of the medium during germination, 2-cc. portions of the various solutions of a series were placed under aseptic conditions in sterile test-tubes. The tubes had a volume of 22 cc. and were plugged with cotton. Several loops of spores were placed in each test-tube of solution, care being exercised to secure a heavy and uniform suspension, and, following inoculation, the cultures were allowed to incubate at a provisional optimum temperature for a period of 20 hours. All of the cultures were set up in duplicate and controls were run simultaneously. The initial and final hydrogen-ion concentrations were determined in the usual way.

Extreme precautions were exercised in the care and the cleaning of glassware. A detailed description of such operations may be found in the writer's earlier paper (Webb, '19).

### EXPERIMENTAL DATA

Cultures were examined at different intervals, depending on

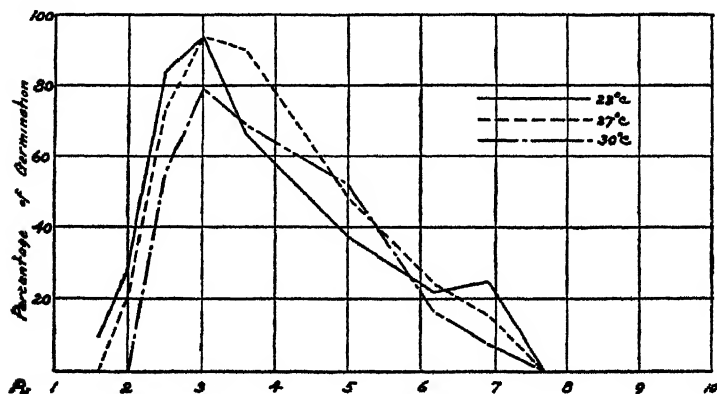


Fig. 2. *Botrytis cinerea* in M/5 mannite solution.

the length of time required for the spores of the particular fungus to germinate, as determined from preliminary experiments. Spore counts were made from 5 different fields of the hanging

drop, involving usually from 50 to 100 spores, and the average percentage of germination recorded. Readings were made with each set of cultures at two different incubation periods, and a third one would have been made had not luxuriant mycelial

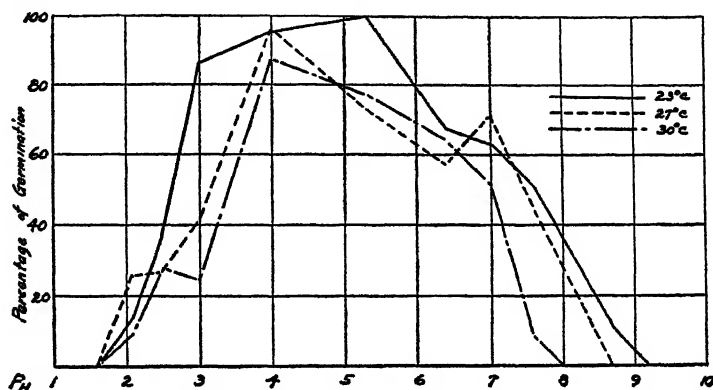


Fig. 3. *Botrytis cinerea* in 2 per cent bacto-peptone solution.

growth in the nutrient solutions prevented. Values obtained from the second reading are considered as reliable as those that might have been obtained from a third reading; in fact, data

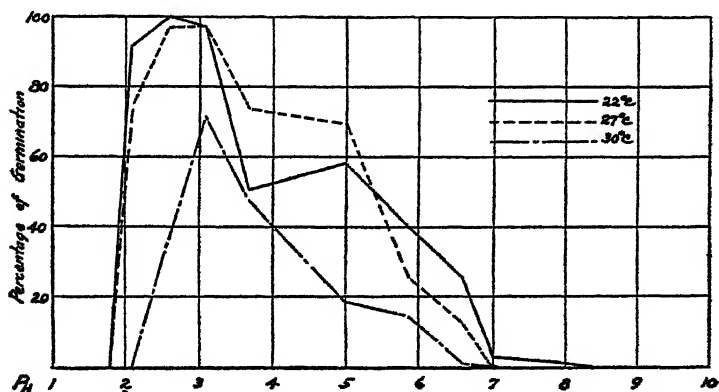


Fig. 4. *Botrytis cinerea* in Czapek's full nutrient solution.

which were accumulated in the preliminary study with incubation periods as denoted by second and third readings showed close agreement, indicating, therefore, that germination was sufficiently and satisfactorily completed at this period. As might be ex-



pected, fluctuations sometimes apparently erratic and seemingly unexplainable occurred occasionally. In such cases, the experiments were always duplicated and frequently triplicated.

The curves are developed from the percentage averages, as indicated, each curve representing the final reading of the germination quantities of a particular organism at a definite temperature in a certain medium. The percentages of spore germination are plotted as ordinates and the hydrogen-ion concentration of the solutions as abscissae. Curves are shown giving germination percentages at each temperature and with each medium. In addition an assembled graph is presented, this being made by averaging the germination percentages of the three temperatures with each of the media.

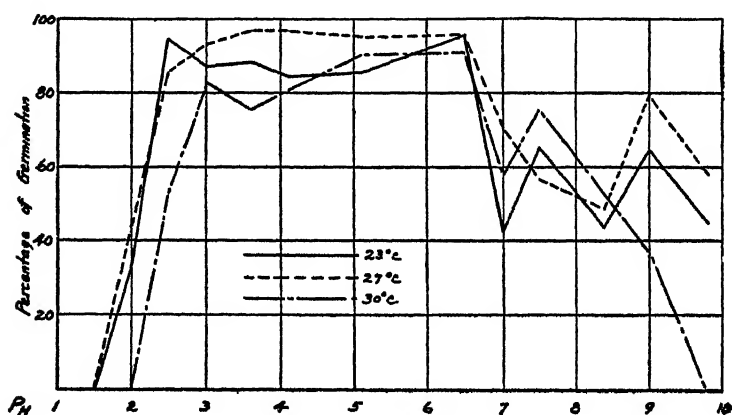


Fig. 5. *Botrytis cinerea* in sugar beet decoction.

In examining the experimental results, it must be borne in mind that perfect germination is not to be expected with these fungi in water or in solutions containing mannite and the acid or the alkali as the only nutrients. Dextrose, in fact, would have yielded higher germination percentages than did mannite, but it is not certain that it would remain stable with the treatment given.

Of the various liquid media employed in this study, the sugar beet decoction has undoubtedly furnished the most interesting data. Germination obtained with the acid cultures has not

been unusual, but that obtained with the alkaline cultures has been most striking.

The spores of *B. cinerea* germinate in the mannite medium within the range  $P_H$  1.6–6.9 with best germination afforded at  $P_H$  3.0 and similar data are obtained in Czapek's full nutrient solution. The relations in the peptone solution are similar, except that there is a slight shift of the germination-acidity curve towards alkalinity. Decidedly different data are furnished with the sugar beet decoction, maximum germination extending in a zone between  $P_H$  3.0 and  $P_H$  6.5 and ranging on the alkaline side beyond  $P_H$  9.6. Germination in "water HCl or KOH" agrees with that in the sugar beet decoction (though not stimulated to such a great extent) in that germination freely occurs under conditions of active alkalinity. This is in direct contrast with the other media employed where no or relatively feeble germination is obtained under such conditions. The relations in "water  $H_3PO_4$  and NaOH" are very similar to those in the mannite solution, but the composition of the two media, it must be remembered, is the same except for the presence and absence of mannite.

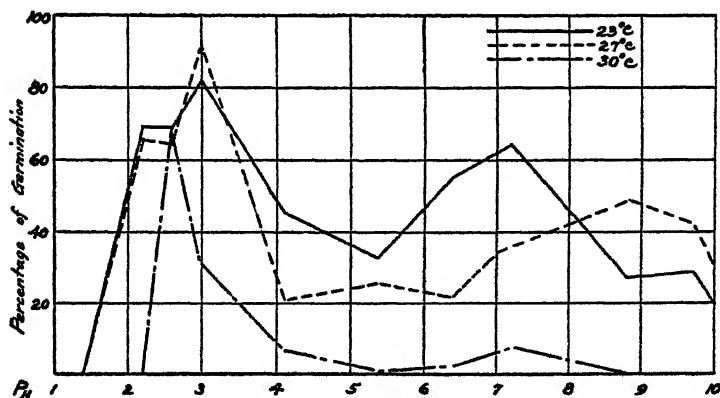


Fig. 6. *Botrytis cinerea* in "water HCl or KOH."

*Aspergillus niger* furnishes germination curves which are very consistent in solutions of mannite, Czapek's solution, and peptone. These relations are similar to those of *Botrytis* and clearly demonstrate the importance of active acidity for germination in these solutions.

The spores of *Lenzites saepiaria* germinate freely in acid media, and this is the only case where the sugar beet decoction fails to stimulate germination in the alkaline cultures.

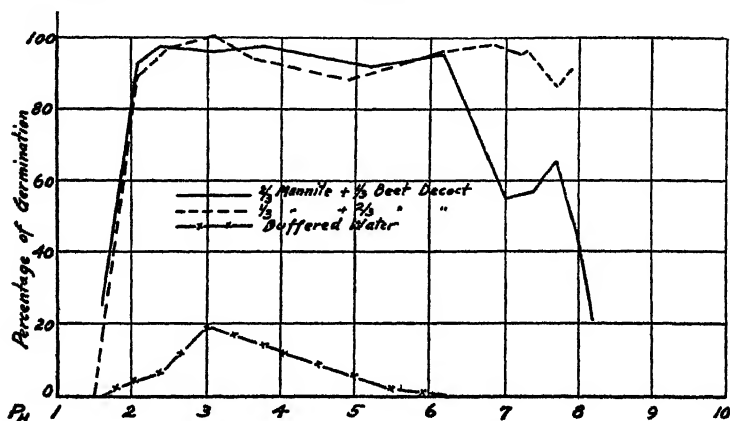


Fig. 7. *Botrytis cinerea* in (1) a medium composed of M/5 mannite solution and sugar beet decoction and (2) "water  $H_3PO_4$  and NaOH" ( $25^\circ C.$ ).

*Penicillium cyclopium* does not appear to be as dependent upon the stimulating effects of hydrogen ions as *Aspergillus* and *Botrytis*, and the same to a greater degree may be said of

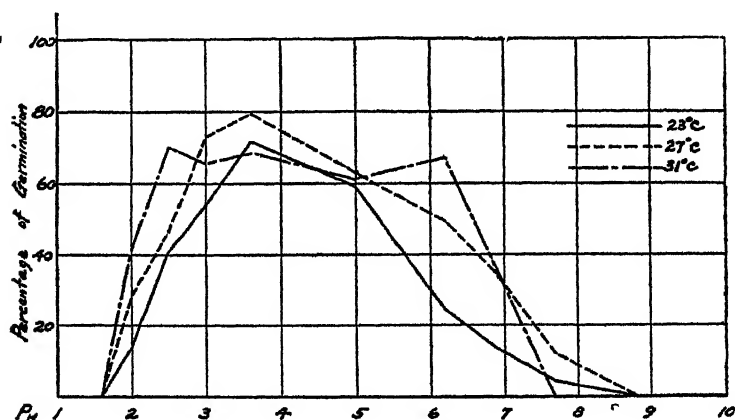


Fig. 8. *Aspergillus niger* in M/5 mannite solution.

*Fusarium* sp. The 2 organisms referred to, *Penicillium* and *Fusarium*, are conspicuous for their double maxima; one on the acid side, and the other near neutrality.

Relatively high active acidity in the synthetic culture media favors spore germination of *Penicillium italicum*, but slightly alkaline conditions in the sugar beet decoction are equally as favorable, if not more so, than acid conditions in the same medium.

In general, a slightly acid or neutral reaction affords most perfect germination of the uredospores of *Puccinia graminis*.

Germination of the spores of *Colletotrichum Gossypii* is very variable and no definite curve is produced, but an alkaline reaction of the medium is most favorable.

The effects of hydrogen-ion concentration upon the germination of these fungous spores in the different culture media are shown in tables I-IX, and such relations are graphically presented in figs. 2-39. Tables I to IX follow.

TABLE I

BOTRYTIS CINEREA. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

| Temp.  | Hrs. | Hydrogen-ion concentration, P <sub>H</sub> |      |      |       |      |      |      |      |      |     |     |       | Media   |
|--------|------|--|------|------|-------|------|------|------|------|------|-----|-----|-------|---------|
|        |      | 1.6  | 2.0  | 2.5  | 3.0   | 3.6  | 5.0  | 6.2  | 6.9  | 7.7  | 8.8 | 9.6 | 10.0+ |         |
| 23° C. | 6    | 0.0  | 0.0  | 29.4 | 63.8  | 41.9 | 26.9 | 14.4 | 1.9  | 0.0  | 0.0 | 0.0 | 0.0   | Mannite |
|        | 20   | 9.5  | 28.0 | 83.5 | 93.1  | 66.0 | 37.5 | 21.9 | 24.5 | 0.0  | 0.0 | 0.0 | 0.0   |         |
| 27° C. | 6    | 0.0  | 0.0  | 38.3 | 65.1  | 75.2 | 33.0 | 10.3 | 1.5  | 0.0  | 0.0 | 0.0 | 0.0   |         |
|        | 20   | 0.0  | 21.0 | 72.3 | 93.6  | 89.5 | 48.1 | 24.0 | 15.6 | 0.0  | 0.0 | 0.0 | 0.0   |         |
| 30° C. | 6    | 0.0  | 0.0  | 0.0  | 17.0  | 6.0  | 4.2  | 1.0  | 0.0  | 0.0  | 0.0 | 0.0 | 0.0   |         |
|        | 20   | 0.0  | 0.0  | 54.9 | 79.1  | 68.4 | 52.1 | 16.3 | 7.6  | 0.0  | 0.0 | 0.0 | 0.0   |         |
| 22° C. | 6    | 0.0  | 0.0  | 88.0 | 93.5  | 91.0 | 44.5 | 66.0 | 14.5 | 22.0 | 0.0 | 0.0 | 0.0   | Czapek  |
|        | 20   | 0.0  | 0.0  | 91.1 | 100.0 | 96.5 | 50.4 | 58.3 | 39.5 | 25.8 | 2.6 | 1.2 | 0.0   |         |
| 27° C. | 6    | 0.0  | 0.0  | 6.5  | 89.0  | 90.0 | 70.5 | 58.0 | 5.0  | 4.0  | 0.0 | 0.0 | 0.0   |         |
|        | 20   | 0.0  | 0.0  | 74.0 | 96.7  | 96.8 | 73.7 | 69.3 | 25.1 | 12.2 | 0.0 | 0.0 | 0.0   |         |
| 30° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0   | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0 | 0.0 | 0.0   |         |
|        | 20   | 0.0  | 0.0  | 0.0  | 36.3  | 71.3 | 47.7 | 18.4 | 14.5 | 1.0  | 0.0 | 0.0 | 0.0   |         |

TABLE I (cont.)

| Temp.  | Hrs. | Hydrogen-ion concentration $P_H$ |       |      |       |      |       |      |      |      |      |      |       |      | Media   |  |
|--------|------|----------------------------------|-------|------|-------|------|-------|------|------|------|------|------|-------|------|---|--|
|        |      | 1.6                              | 2.1   | 2.5  | 3.0   | 4.0  | 5.3   | 6.4  | 7.0  | 7.6  | 8.7  | 9.2  | 10.0+ |      |   |  |
| 23° C. | 6    | 0.0                              | 25.0  | 39.5 | 74.4  | 65.0 | 69.5  | 44.0 | 36.0 | 4.1  | 0.0  | 0.0  | 0.0   |      | Peptone   |  |
|        | 20   | 0.0                              | 23.2  | 36.2 | 86.3  | 95.5 | 100.0 | 67.6 | 63.3 | 51.3 | 11.3 | 0.0  | 0.0   |      |   |  |
| 27° C. | 6    | 0.0                              | 26.8  | 26.3 | 48.0  | 63.0 | 55.5  | 36.2 | 49.2 | 10.0 | 0.0  | 0.0  | 0.0   |      |   |  |
|        | 20   | 0.0                              | 25.4  | 26.1 | 41.7  | 96.0 | 73.6  | 57.0 | 71.2 | 44.3 | 0.0  | 0.0  | 0.0   |      |   |  |
| 30° C. | 6    | 0.0                              | 0.0   | 29.0 | 26.0  | 70.0 | 58.9  | 50.3 | 36.2 | 5.1  | 0.0  | 0.0  | 0.0   |      |   |  |
|        | 20   | 0.0                              | 9.2   | 27.5 | 24.4  | 87.2 | 77.5  | 64.1 | 51.0 | 8.4  | 0.0  | 0.0  | 0.0   |      |   |  |
| 23° C. | 6    | 1.5                              | 2.0   | 2.5  | 3.0   | 3.6  | 4.1   | 5.1  | 6.5  | 7.0  | 7.5  | 8.4  | 9.0   | 9.8  | Beet decoction  |  |
|        | 20   | 0.0                              | 0.0   | 46.0 | 61.3  | 73.0 | 70.8  | 73.4 | 76.2 | 28.1 | 63.3 | 41.3 | 2.0   | 1.0  |   |  |
| 27° C. | 6    | 0.0                              | 32.7  | 94.0 | 86.6  | 88.0 | 84.0  | 85.0 | 94.9 | 42.2 | 65.1 | 43.1 | 64.2  | 44.5 |   |  |
|        | 20   | 0.0                              | 2.8   | 83.3 | 85.1  | 84.4 | 89.8  | 98.0 | 87.4 | 37.1 | 54.0 | 46.9 | 17.0  | 0.5  |   |  |
| 30° C. | 6    | 0.0                              | 43.1  | 85.5 | 92.9  | 96.9 | 96.9  | 95.0 | 95.3 | 70.0 | 56.7 | 48.2 | 79.2  | 58.0 |   |  |
|        | 20   | 0.0                              | 0.0   | 0.0  | 0.0   | 0.0  | 16.8  | 0.0  | 33.4 | 13.7 | 14.2 | 0.0  | 0.0   | 0.0  |   |  |
| 23° C. | 6    | 1.4                              | 2.2   | 2.6  | 3.0   | 4.1  | 5.4   | 6.4  | 7.2  | 8.8  | 9.7  | 10+  |       |      | Water<br>(HCl or KOH)   |  |
|        | 20   | 0.0                              | 59.1  | 68.8 | 74.8  | 19.8 | 23.0  | 53.2 | 31.0 | 8.0  | 7.2  | 5.0  |       |      |   |  |
| 27° C. | 6    | 0.0                              | 69.5  | 69.5 | 82.0  | 45.6 | 32.5  | 55.8 | 64.9 | 26.9 | 28.6 | 20.0 |       |      |   |  |
|        | 20   | 0.0                              | 63.0  | 69.0 | 92.4  | 6.2  | 24.1  | 8.4  | 23.2 | 36.5 | 31.0 | 10.0 |       |      |   |  |
| 30° C. | 6    | 0.0                              | 65.9  | 64.3 | 92.0  | 20.6 | 25.7  | 21.9 | 34.0 | 49.0 | 42.5 | 31.0 |       |      |   |  |
|        | 20   | 0.0                              | 0.0   | 0.0  | 9.0   | 0.0  | 0.0   | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |       |      |   |  |
| 24° C. | 6    | 1.0                              | 2.0   | 2.4  | 3.0   | 4.0  | 5.5   | 6.2  | 7.0  | 7.7  | 8.1  | 8.2  | 8.6   |      | Water<br>( $H_2PO_4$<br>and NaOH)                             |  |
|        | 20   | 0.0                              | 0.0   | 3.3  | 18.4  | 9.1  | 0.0   | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0   |      |   |  |
| 24° C. | 6    | 0.0                              | 3.5   | 6.1  | 18.7  | 12.5 | 1.5   | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0   |      |   |  |
|        | 20   | 0.0                              | 0.0   | 0.0  | 0.0   | 0.0  | 0.0   | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0   |      |   |  |
| 24° C. | 6    | 1.6                              | 2.1   | 2.4  | 3.1   | 3.8  | 5.2   | 6.2  | 7.0  | 7.4  | 7.7  | 8.0  | 8.2   |      |   |  |
|        | 20   | 0.0                              | 45.0  | 89.1 | 93.4  | 90.8 | 86.6  | 91.6 | 18.8 | 8.2  | 6.2  | 1.2  | 0.0   |      |   |  |
| 24° C. | 6    | 25.1                             | 192.0 | 97.8 | 95.9  | 97.5 | 91.7  | 95.0 | 55.3 | 57.1 | 65.0 | 43.8 | 20.7  |      | $\frac{1}{2}$ mannite<br>+<br>$\frac{1}{2}$ B. decoc-<br>tion |  |
|        | 20   | 0.0                              | 88.4  | 96.4 | 100.0 | 94.0 | 88.0  | 96.0 | 98.0 | 94.7 | 96.0 | 86.0 | 90.7  |      |   |  |

TABLE II

ASPERGILLUS NIGER. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

| Temp.  | Hrs. | Hydrogen-ion concentration, P <sub>H</sub> |       |       |       |       |       |       |       |       |       |       |       |                | Media          |
|--------|------|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------------|----------------|
|        |      | 1.6  | 2.0   | 2.5   | 3.0   | 3.6   | 5.0   | 6.2   | 6.9   | 7.7   | 8.8   | 9.6   | 10.0+ |                |                |
| 23° C. | 10   | 0.0  | 0.0   | 30.9  | 43.5  | 14.5  | 8.6   | 4.5   | 1.2   | 0.0   | 0.0   | 0.0   | 0.0   | Mannite        |                |
|        | 20   | 0.0  | 13.2  | 40.7  | 53.9  | 71.7  | 58.9  | 24.0  | 13.6  | 4.0   | 0.0   | 0.0   | 0.0   |                |                |
| 27° C. | 10   | 0.0  | 24.8  | 41.3  | 65.0  | 55.9  | 61.0  | 41.2  | 20.9  | 2.9   | 0.0   | 0.0   | 0.0   |                |                |
|        | 20   | 0.0  | 27.5  | 46.0  | 72.9  | 79.6  | 62.6  | 48.8  | 34.5  | 12.0  | 0.0   | 0.0   | 0.0   |                |                |
| 31° C. | 10   | 0.0  | 22.8  | 56.0  | 55.9  | 67.0  | 52.2  | 67.5  | 24.5  | 0.0   | 0.0   | 0.0   | 0.0   |                |                |
|        | 20   | 0.0  | 41.0  | 70.0  | 65.9  | 68.6  | 61.0  | 67.5  | 36.8  | 0.0   | 0.0   | 0.0   | 0.0   |                |                |
|        |      | 1.2  | 1.8   | 2.1   | 2.6   | 3.1   | 3.7   | 5.0   | 5.9   | 6.6   | 7.0   | 7.9   | 8.4   |                | 9.4            |
| 22° C. | 16   | 0.0  | 0.0   | 5.5   | 34.5  | 72.0  | 84.0  | 38.5  | 60.0  | 38.5  | 36.5  | 15.0  | 5.5   | 0.0            | Czapek         |
|        | 26   | 0.0  | 0.0   | 12.3  | 29.9  | 71.7  | 89.2  | 41.2  | 67.1  | 45.1  | 42.5  | 27.4  | 6.2   | 0.0            |                |
| 27° C. | 16   | 0.0  | 0.0   | 23.0  | 32.0  | 72.0  | 96.5  | 39.5  | 41.0  | 22.0  | 25.0  | 10.5  | 0.0   | 0.0            |                |
|        | 26   | 0.0  | 0.0   | 27.7  | 50.1  | 88.0  | 96.0  | 39.0  | 68.0  | 30.0  | 49.7  | 12.0  | 2.0   | 0.0            |                |
| 31° C. | 16   | 0.0  | 0.0   | 22.5  | 24.5  | 74.0  | 85.0  | 51.0  | 65.0  | 51.0  | 42.0  | 7.5   | 0.0   | 0.0            |                |
|        | 26   | 0.0  | 0.0   | 33.9  | 50.1  | 88.8  | 81.9  | 48.3  | 71.7  | 53.2  | 50.9  | 14.8  | 0.0   | 0.0            |                |
|        |      | 1.6  | 2.1   | 2.5   | 3.0   | 4.0   | 5.3   | 6.4   | 7.0   | 7.6   | 8.7   | 9.2   | 10.0+ |                |                |
| 23° C. | 10   | 0.0  | 0.0   | 0.0   | 0.0   | 32.9  | 8.8   | 0.0   | 0.0   | 0.0   | 0.0   | 0.0   | 0.0   | 0.0            | Peptone        |
|        | 20   | 0.0  | 26.3  | 26.6  | 44.8  | 78.7  | 32.8  | 4.8   | 5.6   | 0.5   | 0.0   | 0.0   | 0.0   | 0.0            |                |
| 27° C. | 10   | 0.0  | 0.0   | 0.0   | 5.7   | 62.6  | 35.9  | 34.1  | 3.4   | 0.0   | 0.0   | 0.0   | 0.0   |                |                |
|        | 20   | 0.0  | 29.1  | 31.0  | 71.0  | 66.1  | 37.3  | 44.2  | 6.6   | 4.4   | 1.0   | 0.0   | 0.0   |                |                |
| 31° C. | 10   | 0.0  | 41.4  | 86.0  | 97.3  | 87.4  | 58.0  | 56.3  | 66.0  | 24.0  | 0.0   | 0.0   | 0.0   |                |                |
|        | 20   | 6.6  | 89.5  | 97.5  | 100.0 | 93.1  | 66.8  | 54.7  | 65.5  | 27.0  | 4.0   | 0.0   | 0.0   |                |                |
|        |      | 1.5  | 2.0   | 2.5   | 3.0   | 3.6   | 4.1   | 5.1   | 6.5   | 7.0   | 7.5   | 8.4   | 9.0   | 9.8            |                |
| 23° C. | 10   | 0.0  | 0.0   | 4.8   | 0.5   | 4.9   | 10.0  | 10.0  | 15.7  | 25.3  | 12.8  | 1.0   | 0.0   | 0.0            | Beet decoction |
|        | 20   | 66.2                                       | 90.9  | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 85.9  | 70.5  | 30.8           |                |
| 27° C. | 10   | 0.0  | 82.5  | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 82.0  | 43.1           |                |
|        | 20   | 20.0                                       | 95.0  | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 69.0           |                |
| 31° C. | 10   | 19.3                                       | 15.4  | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.6  | 0.0            |                |
|        | 20   | 45.0                                       | 62.4  | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 45.2           |                |
|        |      | 1.6  | 2.1   | 2.4   | 3.1   | 3.8   | 5.2   | 6.2   | 7.0   | 7.4   | 7.7   | 8.0   | 8.2   |                |                |
| 24° C. | 10   | 6.2  | 50.6  | 87.5  | 88.5  | 83.4  | 85.3  | 88.8  | 88.8  | 65.9  | 57.4  | 60.0  | 19.3  | ¾ mannite      |                |
|        | 20   | 91.7                                       | 92.9  | 94.0  | 98.6  | 97.3  | 98.4  | 98.0  | 98.0  | 98.0  | 96.5  | 96.0  | 72.6  | ½ B. decoction |                |
|        |      | 1.5  | 2.1   | 2.5   | 3.1   | 3.6   | 4.0   | 6.2   | 6.8   | 7.2   | 7.7   | 7.9   |       | ¾ mannite      |                |
| 24° C. | 10   | 0.0  | 6.0   | 16.0  | 31.0  | 30.4  | 29.3  | 25.6  | 15.0  | 28.4  |       | 10.0  | 6.7   | ½ B. decoction |                |
|        | 20   | 96.0                                       | 100.0 | 100.0 | 96.7  | 100.0 | 92.4  | 100.0 | 100.0 | 96.0  |       | 98.0  | 97.2  |                |                |

TABLE III

LENZITES SAEPIARIA. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

| Temp   | Hrs. | Hydrogen-ion concentration, P <sub>H</sub> |      |      |      |      |      |      |      |      |      |     |       | Media          |
|--------|------|--|------|------|------|------|------|------|------|------|------|-----|-------|----------------|
|        |      | 1.6  | 2.0  | 2.5  | 3.0  | 3.6  | 5.0  | 6.2  | 6.9  | 7.7  | 8.8  | 9.6 | 10.0+ |                |
| 23° C. | 18   | 0.0  | 0.0  | 6.0  | 43.3 | 57.9 | 12.2 | 32.7 | 11.7 | 0.0  | 0.0  | 0.0 | 0.0   | Mannite        |
|        | 30   | 0.0  | 0.0  | 36.8 | 71.8 | 57.1 | 53.3 | 44.1 | 13.8 | 0.0  | 0.0  | 0.0 | 0.0   |                |
| 27° C. | 18   | 0.0  | 0.0  | 10.9 | 48.1 | 58.4 | 49.3 | 34.1 | 12.2 | 0.0  | 0.0  | 0.0 | 0.0   |                |
|        | 30   | 0.0  | 0.0  | 51.7 | 72.1 | 66.3 | 55.0 | 36.8 | 16.8 | 0.0  | 0.0  | 0.0 | 0.0   |                |
| 32° C. | 18   | 0.0  | 0.0  | 36.7 | 62.5 | 31.1 | 21.5 | 24.8 | 10.9 | 0.0  | 0.0  | 0.0 | 0.0   |                |
|        | 30   | 0.0  | 0.0  | 49.0 | 60.0 | 38.6 | 31.0 | 32.0 | 17.2 | 0.0  | 0.0  | 0.0 | 0.0   |                |
| 20° C. | 18   | 0.0  | 0.0  | 0.0  | 6.4  | 41.9 | 41.7 | 30.2 | 14.4 | 8.3  | 0.0  | 0.0 | 0.0   | Czapek         |
|        | 30   | 0.0  | 0.0  | 0.0  | 28.0 | 52.5 | 44.7 | 32.9 | 23.0 | 15.2 | 0.0  | 0.0 | 0.0   |                |
| 25° C. | 18   | 0.0  | 0.0  | 0.0  | 1.0  | 38.2 | 21.9 | 22.6 | 6.9  | 5.7  | 0.0  | 0.0 | 0.0   |                |
|        | 30   | 0.0  | 0.0  | 0.0  | 1.0  | 53.9 | 22.3 | 22.2 | 11.1 | 6.6  | 0.0  | 0.0 | 0.0   |                |
| 30° C. | 18   | 0.0  | 0.0  | 0.0  | 10.5 | 34.9 | 33.9 | 10.0 | 8.2  | 0.0  | 0.0  | 0.0 | 0.0   |                |
|        | 30   | 0.0  | 0.0  | 0.0  | 22.1 | 40.6 | 31.6 | 13.4 | 11.4 | 0.0  | 0.0  | 0.0 | 0.0   |                |
| 23° C. | 18   | 0.0  | 0.0  | 51.7 | 52.4 | 46.7 | 26.7 | 10.0 | 1.9  | 0.0  | 0.0  | 0.0 | 0.0   | Peptone        |
|        | 30   | 0.0  | 8.9  | 90.8 | 91.0 | 59.2 | 36.3 | 13.8 | 2.0  | 0.0  | 0.0  | 0.0 | 0.0   |                |
| 27° C. | 18   | 0.0  | 0.0  | 58.8 | 70.7 | 70.2 | 23.9 | 20.9 | 4.0  | 0.0  | 0.0  | 0.0 | 0.0   |                |
|        | 30   | 0.0  | 13.4 | 84.9 | 94.4 | 82.0 | 42.3 | 31.7 | 6.0  | 0.0  | 0.0  | 0.0 | 0.0   |                |
| 32° C. | 18   | 0.0  | 0.0  | 27.5 | 40.5 | 32.3 | 26.9 | 18.6 | 3.0  | 0.0  | 0.0  | 0.0 | 0.0   |                |
|        | 30   | 0.0  | 51.8 | 82.9 | 92.3 | 70.0 | 29.0 | 25.0 | 3.8  | 0.0  | 0.0  | 0.0 | 0.0   |                |
| 23° C. | 18   | 0.0  | 28.6 | 95.0 | 88.0 | 73.8 | 85.0 | 40.0 | 41.2 | 85.6 | 70.0 | 0.0 | 0.0   | Beet decoction |
|        | 30   | 0.0  | 76.6 | 56.6 | 78.9 | 84.5 | 79.8 | 39.5 | 35.6 | 8.9  | 76.0 | 0.0 | 0.0   |                |
| 27° C. | 18   | 0.0  | 76.6 | 56.6 | 78.9 | 84.5 | 79.8 | 39.5 | 35.6 | 8.9  | 76.0 | 0.0 | 0.0   |                |
|        | 30   | 0.0  | 76.6 | 56.6 | 78.9 | 84.5 | 79.8 | 39.5 | 35.6 | 8.9  | 76.0 | 0.0 | 0.0   |                |
| 31° C. | 18   | 0.0  | 17.7 | 47.5 | 93.8 | 62.7 | 51.0 | 35.7 | 43.7 | 70.9 | 37.5 | 0.0 | 0.0   | Beet decoction |
|        | 30   | 0.0  | 17.7 | 47.5 | 93.8 | 62.7 | 51.0 | 35.7 | 43.7 | 70.9 | 37.5 | 0.0 | 0.0   |                |

TABLE IV

PENICILLIUM CYCLOPIUM. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

| Temp.  | Hrs. | Hydrogen-ion concentration, P <sub>H</sub> |      |      |      |       |       |       |       |       |       |       |       | Media          |
|--------|------|--|------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|----------------|
|        |      | 1.6  | 2.0  | 2.5  | 3.0  | 3.6   | 5.0   | 6.2   | 6.9   | 7.7   | 8.8   | 9.6   | 10.0+ |                |
| 23° C. | 10   | 0.0  | 0.0  | 0.0  | 0.0  | 41.9  | 43.0  | 53.5  | 38.2  | 11.9  | 0.0   | 0.0   | 0.0   | Mannite        |
|        | 20   | 0.0  | 4.9  | 3.0  | 22.8 | 62.6  | 45.8  | 62.5  | 54.0  | 13.4  | 0.0   | 0.0   | 0.0   |                |
| 27° C. | 10   | 0.0  | 0.0  | 0.0  | 0.0  | 58.3  | 37.1  | 62.4  | 23.7  | 13.0  | 0.0   | 0.0   | 0.0   |                |
|        | 20   | 0.0  | 45.5 | 66.2 | 48.0 | 52.2  | 43.8  | 62.4  | 54.7  | 19.5  | 0.0   | 0.0   | 0.0   |                |
| 31° C. | 10   | 0.0  | 0.0  | 0.0  | 0.0  | 5.5   | 8.0   | 35.1  | 10.9  | 0.0   | 0.0   | 0.0   | 0.0   |                |
|        | 20   | 0.0  | 0.0  | 2.0  | 23.6 | 25.5  | 21.1  | 48.1  | 35.0  | 1.0   | 0.0   | 0.0   | 0.0   |                |
|        |      | 1.2  | 1.8  | 2.1  | 2.6  | 3.1   | 3.7   | 5.0   | 5.9   | 6.6   | 7.0   | 7.9   | 8.4   |                |
| 22° C. | 18   | 0.0  | 0.0  | 0.0  | 48.0 | 85.0  | 85.5  | 93.0  | 80.5  | 91.5  | 91.0  | 74.5  | 30.0  | Czapek         |
|        | 27   | 0.0  | 0.0  | 0.0  | 68.6 | 100.0 | 97.2  | 86.3  | 77.3  | 94.9  | 97.6  | 90.5  | 45.1  |                |
| 27° C. | 18   | 0.0  | 0.0  | 3.5  | 23.5 | 77.0  | 73.0  | 78.5  | 73.5  | 70.0  | 63.5  | 40.5  | 12.0  |                |
|        | 27   | 0.0  | 0.0  | 46.9 | 67.0 | 100.0 | 88.3  | 90.5  | 88.3  | 100.0 | 63.5  | 49.0  | 24.1  |                |
| 31° C. | 18   | 0.0  | 0.0  | 0.0  | 0.0  | 22.0  | 68.0  | 37.5  | 15.5  | 10.0  | 16.0  | 15.0  | 0.0   |                |
|        | 27   | 0.0  | 0.0  | 0.0  | 32.9 | 66.8  | 93.8  | 40.1  | 28.4  | 22.2  | 25.9  | 18.7  | 4.2   |                |
|        |      | 1.6  | 2.1  | 2.5  | 3.0  | 4.0   | 5.3   | 6.4   | 7.0   | 7.6   | 8.7   | 9.2   | 10.0+ |                |
| 23° C. | 10   | 0.0  | 0.0  | 0.0  | 2.0  | 32.2  | 45.3  | 46.9  | 58.7  | 45.6  | 9.1   | 4.1   | 0.0   | Peptone        |
|        | 20   | 0.0  | 18.4 | 22.4 | 46.1 | 68.2  | 74.1  | 76.7  | 86.1  | 88.7  | 59.1  | 41.7  | 12.8  |                |
| 27° C. | 10   | 0.0  | 0.0  | 0.0  | 3.7  | 40.0  | 68.4  | 55.1  | 53.2  | 50.9  | 11.7  | 2.1   | 0.0   |                |
|        | 20   | 0.0  | 10.6 | 30.7 | 54.8 | 72.5  | 85.0  | 85.8  | 84.8  | 76.8  | 49.0  | 60.2  | 5.0   |                |
| 31° C. | 10   | 0.0  | 0.0  | 0.0  | 0.0  | 5.5   | 17.9  | 23.8  | 1.6   | 4.0   | 0.5   | 0.0   | 0.0   |                |
|        | 20   | 0.0  | 0.0  | 8.4  | 8.0  | 65.6  | 56.1  | 49.0  | 37.6  | 56.1  | 36.9  | 26.4  | 0.0   |                |
|        |      | 1.5  | 2.0  | 2.5  | 3.0  | 3.6   | 4.1   | 5.1   | 6.5   | 7.0   | 7.5   | 8.4   | 9.0   |                |
| 23° C. | 10   | 0.0  | 0.0  | 0.0  | 0.0  | 1.0   | 60.0  | 50.7  | 52.5  | 42.8  | 35.4  | 17.7  | 20.0  | Beef decoction |
|        | 20   | 0.0  | 0.0  | 45.3 | 78.8 | 92.0  | 100.0 | 98.0  | 99.5  | 100.0 | 98.0  | 100.0 | 100.0 |                |
| 27° C. | 10   | 0.0  | 0.0  | 0.0  | 2.0  | 50.6  | 81.9  | 89.1  | 46.7  | 23.9  | 27.0  | 62.4  | 43.2  |                |
|        | 20   | 0.0  | 0.0  | 39.6 | 79.2 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |                |
| 31° C. | 10   | 0.0  | 0.0  | 0.0  | 37.5 | 55.2  | 73.5  | 48.4  | 70.6  | 38.9  | 49.0  | 73.8  | 76.3  |                |
|        | 20   | 0.0  | 0.0  | 0.0  | 55.0 | 86.3  | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 61.3  |                |



TABLE V

PENICILLIUM ITALICUM. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

| Temp.  | Hrs. | Hydrogen-ion concentration, $P_H$ |      |      |      |      |      |      |      |      |      |      |       | Media   |                |
|--------|------|-----------------------------------|------|------|------|------|------|------|------|------|------|------|-------|---------|----------------|
|        |      | 1.6                               | 2.0  | 2.5  | 3.0  | 3.6  | 5.0  | 6.2  | 6.9  | 7.7  | 8.8  | 9.6  | 10.0+ |         |                |
| 15° C. | 20   | 0.0                               | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0   | Mannite |                |
|        | 35   | 0.0                               | 0.0  | 0.5  | 5.4  | 4.2  | 0.0  | 0.0  | 0.0  | 3.5  | 0.0  | 0.0  | 0.0   |         |                |
| 23° C. | 20   | 0.0                               | 3.3  | 1.1  | 5.2  | 3.7  | 1.0  | 0.0  | 1.0  | 0.0  | 0.0  | 0.0  | 0.0   |         |                |
|        | 35   | 0.0                               | 12.3 | 4.0  | 7.7  | 6.0  | 2.0  | 1.0  | 1.8  | 15.7 | 0.0  | 0.0  | 0.0   |         |                |
| 27° C. | 20   | 0.0                               | 0.0  | 4.5  | 6.0  | 5.7  | 0.0  | 0.0  | 5.0  | 0.0  | 0.0  | 0.0  | 0.0   |         |                |
|        | 35   | 0.0                               | 1.0  | 6.1  | 8.0  | 12.9 | 3.5  | 2.0  | 7.5  | 20.0 | 0.0  | 0.0  | 0.0   |         |                |
|        |      | 1.2                               | 1.8  | 2.1  | 2.6  | 3.2  | 3.7  | 4.8  | 6.0  | 6.6  | 7.2  | 8.0  | 8.2   | 9.0     |                |
| 15° C. | 20   | 0.0                               | 0.0  | 0.0  | 4.0  | 2.5  | 0.0  | 0.0  | 0.0  | 0.0  | 1.7  | 0.0  | 0.0   | 0.0     | Czapek         |
|        | 35   | 0.0                               | 0.0  | 20.0 | 38.0 | 47.6 | 38.2 | 4.6  | 26.7 | 18.7 | 2.5  | 0.5  | 0.3   | 0.0     |                |
| 21° C. | 20   | 0.0                               | 7.5  | 21.5 | 44.5 | 25.3 | 19.5 | 2.0  | 3.9  | 5.5  | 2.2  | 0.0  | 0.0   | 0.0     |                |
|        | 35   | 0.0                               | 55.4 | 58.2 | 45.5 | 42.7 | 45.3 | 33.2 | 39.9 | 23.7 | 4.5  | 3.5  | 1.5   | 0.0     |                |
| 26° C. | 20   | 0.0                               | 0.0  | 0.0  | 32.4 | 5.0  | 9.2  | 2.5  | 9.4  | 11.5 | 1.3  | 0.0  | 0.0   | 0.0     |                |
|        | 35   | 0.0                               | 22.4 | 22.9 | 56.9 | 33.3 | 30.5 | 25.4 | 40.9 | 17.7 | 2.8  | 3.0  | 0.0   | 0.0     |                |
|        |      | 1.6                               | 2.1  | 2.5  | 3.0  | 4.0  | 5.5  | 6.6  | 7.2  | 7.6  | 8.7  | 9.1  | 10.0+ |         |                |
| 15° C. | 10   | 0.0                               | 0.0  | 15.3 | 42.4 | 55.2 | 9.5  | 4.3  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0   | Peptone |                |
|        | 20   | 22.9                              | 20.9 | 31.0 | 76.6 | 90.9 | 35.4 | 24.8 | 0.0  | 0.0  | 0.0  | 0.0  | 0.0   |         |                |
| 23° C. | 10   | 0.0                               | 0.0  | 6.4  | 8.1  | 34.8 | 11.6 | 8.8  | 2.6  | 0.0  | 0.0  | 0.0  | 0.0   |         |                |
|        | 20   | 14.0                              | 22.7 | 89.2 | 89.6 | 91.6 | 46.0 | 40.5 | 13.0 | 0.0  | 0.0  | 0.0  | 0.0   |         |                |
| 27° C. | 10   | 0.0                               | 0.0  | 15.0 | 21.9 | 65.8 | 21.0 | 13.7 | 4.5  | 0.0  | 0.0  | 0.0  | 0.0   |         |                |
|        | 20   | 3.7                               | 3.8  | 79.4 | 85.5 | 82.9 | 58.6 | 46.1 | 16.6 | 0.0  | 0.0  | 0.0  | 0.0   |         |                |
|        |      | 1.8                               | 2.4  | 2.7  | 3.1  | 3.6  | 4.2  | 5.0  | 5.8  | 6.0  | 6.8  | 8.0  | 8.6   | 8.9     |                |
| 15° C. | 20   | 0.0                               | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0   | 0.0     | Beet decoction |
|        | 35   | 0.0                               | 47.5 | 43.5 | 49.9 | 49.4 | 41.7 | 57.1 | 41.0 | 57.4 | 71.6 | 74.1 | 63.8  | 10.3    |                |
| 20° C. | 20   | 0.0                               | 13.5 | 14.5 | 8.9  | 31.8 | 37.8 | 49.9 | 42.8 | 52.8 | 45.1 | 10.9 | 2.2   | 0.0     |                |
|        | 35   | 0.0                               | 51.3 | 50.5 | 47.4 | 50.2 | 61.4 | 67.0 | 51.9 | 48.4 | 71.5 | 65.4 | 52.8  | 59.1    |                |
| 25° C. | 20   | 0.0                               | 5.6  | 8.0  | 36.8 | 65.0 | 47.1 | 46.0 | 42.0 | 60.7 | 60.0 | 54.0 | 28.0  | 0.0     |                |
|        | 35   | 0.0                               | 49.6 | 30.4 | 48.3 | 64.6 | 58.4 | 54.4 | 44.3 | 57.3 | 63.8 | 60.6 | 60.0  | 15.0    |                |

TABLE VI

PUCCINIA GRAMINIS. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

| Temp.  | Hrs. | Hydrogen-ion concentration, P <sub>H</sub> |     |      |      |      |      |      |      |      |      |      |       |                    | Media |
|--------|------|--|-----|------|------|------|------|------|------|------|------|------|-------|--------------------|-------|
|        |      | 1.6  | 2.0 | 2.5  | 3.0  | 3.6  | 5.0  | 6.2  | 6.9  | 7.7  | 8.8  | 9.6  | 10.0+ |                    |       |
| 12° C. | 6    | 0.0  | 0.0 | 0.0  | 2.0  | 42.6 | 39.1 | 40.0 | 9.1  | 0.0  | 0.0  | 0.0  | 0.0   | Mannite            |       |
|        | 20   | 0.0  | 0.0 | 0.0  | 2.0  | 58.0 | 52.4 | 59.8 | 15.5 | 0.0  | 0.0  | 0.0  | 0.0   |                    |       |
| 15° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 30.2 | 70.0 | 55.6 | 48.1 | 1.7  | 0.0  | 0.0  | 0.0   |                    |       |
|        | 20   | 0.0  | 0.0 | 2.0  | 16.1 | 41.6 | 75.4 | 54.2 | 55.7 | 2.0  | 0.0  | 0.0  | 0.0   |                    |       |
| 23° C. | 6    | 0.0  | 0.0 | 0.0  | 24.9 | 51.1 | 65.4 | 35.0 | 42.5 | 2.5  | 0.0  | 0.0  | 0.0   |                    |       |
|        | 20   | 0.0  | 0.0 | 0.5  | 50.8 | 57.7 | 67.4 | 39.9 | 51.9 | 6.7  | 0.0  | 0.0  | 0.0   |                    |       |
|        |      | 1.2  | 1.8 | 2.1  | 2.5  | 3.0  | 3.9  | 5.0  | 5.9  | 6.6  | 7.1  | 8.0  | 8.5   | 9.2                |       |
| 12° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 42.0 | 96.5 | 65.0 | 87.5 | 63.5 | 34.0 | 14.0 | 0.0   | Czapek             |       |
|        | 18   | 0.0  | 0.0 | 0.0  | 0.0  | 59.4 | 94.0 | 65.0 | 95.0 | 61.0 | 46.0 | 45.0 | 0.0   |                    |       |
| 16° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 72.5 | 92.0 | 65.0 | 89.5 | 64.5 | 40.5 | 14.5 | 3.5   |                    |       |
|        | 18   | 0.0  | 0.0 | 0.0  | 0.0  | 79.4 | 97.8 | 68.7 | 89.6 | 77.3 | 51.9 | 45.0 | 4.7   |                    |       |
| 22° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 57.0 | 44.5 | 71.0 | 57.5 | 41.5 | 13.5 | 0.0  | 0.0   |                    |       |
|        | 18   | 0.0  | 0.0 | 0.0  | 0.0  | 72.4 | 50.0 | 73.9 | 56.6 | 46.6 | 24.4 | 3.3  | 0.0   |                    |       |
|        |      | 1.6  | 2.1 | 2.5  | 3.0  | 4.0  | 5.5  | 6.6  | 7.2  | 7.6  | 8.7  | 9.1  | 10.0+ |                    |       |
| 12° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 68.4 | 85.2 | 87.4 | 76.5 | 43.2 | 0.0  | 0.0  | 0.0   | Peptone            |       |
|        | 20   | 0.0  | 0.0 | 0.0  | 0.0  | 28.4 | 78.2 | 90.3 | 96.7 | 81.7 | 56.3 | 0.0  | 0.0   |                    |       |
| 15° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 29.2 | 70.5 | 55.6 | 45.0 | 31.2 | 0.0  | 0.0  | 0.0   |                    |       |
|        | 20   | 0.0  | 0.0 | 0.0  | 0.0  | 65.7 | 89.5 | 78.0 | 58.5 | 62.0 | 0.0  | 0.0  | 0.0   |                    |       |
| 23° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 21.4 | 70.2 | 48.8 | 59.1 | 37.9 | 0.0  | 0.0  | 0.0   |                    |       |
|        | 20   | 0.0  | 0.0 | 0.0  | 0.0  | 51.6 | 72.5 | 50.0 | 58.6 | 50.5 | 0.0  | 0.0  | 0.0   |                    |       |
|        |      | 1.5  | 2.0 | 2.5  | 3.0  | 3.6  | 4.1  | 5.1  | 6.5  | 7.0  | 7.5  | 8.4  | 9.0   | 9.8                |       |
| 12° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 36.7 | 71.6 | 50.0 | 46.2 | 63.5 | 69.0 | 63.7 | 0.0   | Beet decoction     |       |
|        | 20   | 0.0  | 0.0 | 0.0  | 10.7 | 37.9 | 77.5 | 55.0 | 45.5 | 79.2 | 74.0 | 75.0 | 13.9  |                    |       |
| 15° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 15.9 | 83.8 | 83.7 | 59.2 | 69.7 | 62.5 | 20.0 | 0.0   |                    |       |
|        | 20   | 0.0  | 0.0 | 0.0  | 0.0  | 18.8 | 82.9 | 85.1 | 65.9 | 81.7 | 65.7 | 50.9 | 25.2  |                    |       |
| 23° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 40.8 | 52.0 | 60.6 | 38.9 | 62.2 | 56.7 | 53.4 | 0.0   |                    |       |
|        | 20   | 0.0  | 0.0 | 0.0  | 0.0  | 38.9 | 52.0 | 66.1 | 42.5 | 71.2 | 72.6 | 52.4 | 0.0   |                    |       |
|        |      | 1.2  | 2.1 | 2.5  | 3.0  | 3.9  | 5.2  | 5.8  | 6.8  | 8.0  | 9.4  |      |       |                    |       |
| 10° C. | 6    | 0.0  | 0.0 | 3.8  | 30.2 | 43.0 | 37.1 | 43.0 | 33.1 | 38.7 | 25.5 |      |       | Water (HCl or KOH) |       |
|        | 18   | 0.0  | 0.0 | 19.6 | 73.4 | 70.6 | 65.2 | 62.5 | 46.0 | 40.8 | 28.0 |      |       |                    |       |
| 16° C. | 6    | 0.0  | 0.0 | 0.0  | 0.0  | 70.3 | 66.6 | 50.2 | 56.9 | 56.7 | 31.4 |      |       |                    |       |
|        | 18   | 0.0  | 0.0 | 0.0  | 8.4  | 72.1 | 73.2 | 53.2 | 68.7 | 57.9 | 30.8 |      |       |                    |       |
| 22° C. | 6    | 0.0  | 0.0 | 19.2 | 48.2 | 55.0 | 49.1 | 41.9 | 34.5 | 39.6 | 25.0 |      |       |                    |       |
|        | 18   | 0.0  | 0.0 | 22.6 | 60.0 | 57.0 | 57.0 | 55.2 | 45.0 | 43.0 | 22.5 |      |       |                    |       |

TABLE VII

FUSARIUM SP. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

| Temp.  | Hrs. | Hydrogen-ion concentration, P <sub>H</sub> |      |      |      |       |       |       |       |       |       |       |       | Media          |
|--------|------|--|------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|----------------|
|        |      | 1.6  | 2.0  | 2.5  | 3.0  | 3.6   | 5.0   | 6.2   | 6.9   | 7.7   | 8.8   | 9.6   | 10.0+ |                |
| 23° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 24.3  | 58.8  | 65.4  | 69.3  | 12.2  | 12.2  | 17.0  | 2.0   | Mannite        |
|        | 20   | 0.0  | 0.0  | 2.5  | 34.0 | 58.8  | 64.2  | 62.0  | 71.3  | 31.9  | 34.1  | 49.0  | 39.0  |                |
| 27° C. | 6    | 0.0  | 0.0  | 0.0  | 1.2  | 29.2  | 33.5  | 47.2  | 51.3  | 8.3   | 0.0   | 1.0   | 0.0   |                |
|        | 20   | 0.0  | 0.0  | 4.4  | 43.2 | 59.8  | 58.8  | 64.8  | 81.0  | 31.2  | 26.3  | 40.9  | 22.7  |                |
| 32° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 13.0  | 27.2  | 30.9  | 28.2  | 0.0   | 0.0   | 0.0   | 0.0   |                |
|        | 20   | 0.0  | 0.0  | 7.5  | 21.6 | 41.4  | 48.7  | 68.8  | 70.0  | 11.3  | 8.6   | 5.2   | 4.9   |                |
|        |      | 1.2  | 1.8  | 2.1  | 2.6  | 3.1   | 3.7   | 5.0   | 5.9   | 6.6   | 7.0   | 7.9   | 8.4   | Czapek         |
| 22° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 79.5  | 69.0  | 67.5  | 82.0  | 83.0  | 54.0  | 72.0  | 91.0  |                |
|        | 20   | 0.0  | 0.0  | 0.0  | 83.2 | 100.0 | 89.7  | 83.4  | 81.5  | 86.2  | 66.3  | 89.4  | 97.8  |                |
| 27° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 58.0  | 86.0  | 83.5  | 83.5  | 86.5  | 78.5  | 93.0  | 92.0  |                |
|        | 20   | 0.0  | 0.0  | 0.0  | 82.6 | 91.9  | 91.9  | 84.3  | 92.4  | 93.7  | 97.2  | 100.0 | 100.0 |                |
| 31° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 81.5  | 72.0  | 65.5  | 67.0  | 83.0  | 61.0  | 82.0  | 89.5  |                |
|        | 20   | 0.0  | 0.0  | 0.0  | 68.8 | 100.0 | 83.9  | 80.8  | 80.0  | 97.8  | 79.5  | 86.7  | 100.0 |                |
|        |      | 1.6  | 2.1  | 2.5  | 3.0  | 4.0   | 5.3   | 6.4   | 7.0   | 7.6   | 8.7   | 9.2   | 10.0+ | Peptone        |
| 23° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 60.2  | 90.5  | 77.5  | 75.8  | 92.8  | 77.4  | 76.9  | 71.9  |                |
|        | 20   | 0.0  | 21.2 | 55.0 | 65.5 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 87.5  | 90.0  | 90.0  |                |
| 27° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 66.3  | 82.7  | 87.2  | 61.7  | 83.7  | 89.3  | 89.5  | 65.2  |                |
|        | 20   | 0.0  | 16.0 | 46.0 | 71.9 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |                |
| 31° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 44.0  | 69.0  | 50.7  | 67.7  | 64.6  | 62.6  | 64.0  | 63.8  |                |
|        | 20   | 0.0  | 8.9  | 10.0 | 55.9 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |                |
|        |      | 1.5  | 2.0  | 2.5  | 3.0  | 3.6   | 4.1   | 5.1   | 6.5   | 7.0   | 7.5   | 8.4   | 9.0   | Beet decoction |
| 23° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 5.0   | 15.0  | 34.2  | 39.6  | 63.8  | 54.6  | 49.9  | 43.3  |                |
|        | 20   | 0.0  | 0.0  | 11.0 | 37.1 | 76.7  | 85.0  | 100.0 | 100.0 | 100.0 | 100.0 | 92.5  | 92.5  |                |
| 27° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 16.0  | 28.5  | 52.1  | 50.0  | 62.6  | 86.7  | 68.0  | 55.0  |                |
|        | 20   | 0.0  | 0.0  | 0.0  | 8.8  | 78.6  | 90.0  | 100.0 | 100.0 | 100.0 | 100.0 | 95.0  | 95.0  |                |
| 31° C. | 6    | 0.0  | 0.0  | 0.0  | 0.0  | 0.0   | 10.0  | 25.0  | 24.0  | 27.0  | 48.7  | 31.3  | 32.9  |                |
|        | 20   | 0.0  | 0.0  | 0.0  | 0.0  | 5.0   | 52.3  | 77.2  | 78.8  | 95.0  | 100.0 | 86.3  | 81.0  |                |

TABLE VII (cont.)

| Temp.  | Hrs. | Hydrogen-ion concentration, $P_H$ |      |      |      |      |      |      |      |      |      |       |      |  | Media   |  |
|--------|------|-----------------------------------|------|------|------|------|------|------|------|------|------|-------|------|--|---|--|
|        |      | 1.4                               | 2.2  | 2.6  | 3.0  | 4.1  | 5.4  | 6.4  | 7.2  | 8.8  | 9.7  | 10.0+ |      |  | Water (HCl or KOH)                              |  |
| 23° C. | 6    | 0.0                               | 0.0  | 0.0  | 69.1 | 36.3 | 29.4 | 62.5 | 51.7 | 55.0 | 47.7 | 36.5  |      |  |   |  |
|        | 20   | 0.0                               | 30.3 | 24.8 | 83.5 | 51.4 | 47.4 | 74.0 | 60.0 | 60.0 | 48.6 | 53.7  |      |  |   |  |
| 27° C. | 6    | 0.0                               | 0.0  | 51.3 | 52.8 | 43.9 | 43.7 | 55.1 | 64.4 | 40.7 | 43.9 | 47.5  |      |  |   |  |
|        | 20   | 0.0                               | 0.0  | 81.1 | 85.2 | 70.7 | 56.5 | 53.3 | 65.0 | 50.8 | 55.2 | 71.3  |      |  |   |  |
| 31° C. | 6    | 0.0                               | 0.0  | 5.0  | 21.6 | 4.7  | 8.2  | 26.5 | 35.4 | 15.3 | 8.9  | 7.0   |      |  |   |  |
|        | 20   | 0.0                               | 13.0 | 12.5 | 30.0 | 14.0 | 11.9 | 59.9 | 47.4 | 22.8 | 19.6 | 30.3  |      |  |   |  |
|        |      | 1.6                               | 2.0  | 2.4  | 3.0  | 4.0  | 5.5  | 6.2  | 7.0  | 7.7  | 8.1  | 8.2   | 8.6  |  | Water (H <sub>2</sub> PO <sub>4</sub> and NaOH) |  |
| 24° C. | 6    | 0.0                               | 0.0  | 0.0  | 2.0  | 37.6 | 38.5 | 27.2 | 24.1 | 27.4 | 22.3 | 10.0  | 8.2  |  |   |  |
|        | 20   | 0.0                               | 13.7 | 20.0 | 27.1 | 76.4 | 76.6 | 58.3 | 68.5 | 73.2 | 41.2 | 40.0  | 15.0 |  |   |  |

TABLE VIII

COLLETOTRICHUM GOSSYPHII. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

| Temp.  | Hrs. | Hydrogen-ion concentration, $P_H$ |     |     |     |      |      |      |      |      |      |      |       |      | Media          |  |
|--------|------|-----------------------------------|-----|-----|-----|------|------|------|------|------|------|------|-------|------|----------------|--|
|        |      | 1.2                               | 1.8 | 2.1 | 2.6 | 3.2  | 3.7  | 4.8  | 6.0  | 6.6  | 7.2  | 8.0  | 8.2   | 9.0  | Czapek         |  |
| 23° C. | 10   | 0.0                               | 0.0 | 0.0 | 0.0 | 18.5 | 6.4  | 11.7 | 23.6 | 15.7 | 16.9 | 9.3  | 11.0  | 5.0  |                |  |
| 27° C. | 10   | 0.0                               | 0.0 | 0.0 | 0.0 | 5.2  | 11.1 | 12.1 | 12.6 | 14.3 | 17.5 | 10.4 | 12.7  | 4.0  |                |  |
| 31° C. | 10   | 0.0                               | 0.0 | 0.0 | 0.0 | 6.7  | 16.6 | 21.2 | 17.6 | 18.7 | 18.3 | 18.0 | 29.0  | 18.9 |                |  |
|        |      | 1.6                               | 2.1 | 2.5 | 3.0 | 4.0  | 5.5  | 6.6  | 7.2  | 7.6  | 8.7  | 9.1  | 10.0+ |      | Peptone        |  |
| 23° C. | 10   | 0.0                               | 0.0 | 0.0 | 0.0 | 0.0  | 28.3 | 14.6 | 21.9 | 24.7 | 25.3 | 0.0  | 0.0   |      |                |  |
| 27° C. | 10   | 0.0                               | 0.0 | 0.0 | 0.0 | 0.0  | 29.4 | 21.2 | 21.8 | 28.4 | 24.4 | 18.0 | 13.6  |      |                |  |
| 31° C. | 10   | 0.0                               | 0.0 | 0.0 | 0.0 | 0.0  | 40.2 | 27.4 | 29.8 | 27.5 | 30.0 | 22.3 | 14.1  |      |                |  |
|        |      | 1.5                               | 2.0 | 2.5 | 3.0 | 3.6  | 4.1  | 5.1  | 6.5  | 7.0  | 7.5  | 8.4  | 9.0   | 9.8  | Beet decoction |  |
| 23° C. | 10   | 0.0                               | 0.0 | 0.0 | 0.0 | 0.0  | 0.5  | 8.9  | 25.3 | 25.0 | 21.9 | 27.3 | 25.7  | 22.6 |                |  |
| 27° C. | 10   | 0.0                               | 0.0 | 0.0 | 0.0 | 0.0  | 3.0  | 11.7 | 39.3 | 25.0 | 22.3 | 19.5 | 24.1  | 35.2 |                |  |
| 31° C. | 10   | 0.0                               | 0.0 | 0.0 | 0.0 | 0.0  | 8.5  | 14.3 | 26.9 | 27.6 | 27.4 | 32.6 | 26.1  | 49.3 |                |  |

TABLE X

## ASSEMBLED DATA

| Organisms                    | Percentage of germination |                   | Hydrogen-ion concentration, P <sub>H</sub> |                   |  | Media  |
|------------------------------|---------------------------|-------------------|--|-------------------|--|--|
|                              | Primary maximum           | Secondary maximum | Primary optimum                            | Secondary optimum | Range of germination                                 |  |
| <i>Aspergillus niger</i>     | 73.3                      | 68.7              | 3.6  | 5.9               | 2.0-7.7  | Mannite  |
|                              | 89.0                      |                   | 3.7  |                   | 2.1-8.4  | Czapek   |
|                              | 79.3                      |                   | 4.0  |                   | *1.6-8.7   | Peptone  |
|                              | 100.0                     |                   | 2.5-7.5                                    |                   | *1.5-9.8*  | Beet decoction                                       |
|                              |                           |                   |  |                   | *1.6-8.2*  | $\frac{3}{8}$ mannite + $\frac{1}{8}$ beet decoction |
|                              |                           |                   |  | *1.5-7.9*         | $\frac{1}{8}$ mannite + $\frac{3}{8}$ beet decoction |  |
| <i>Penicillium cyclopium</i> | 57.7                      | 46.8              | 6.2  | 3.6               | 2.0-7.7  | Mannite  |
|                              | 93.1                      | 72.4              | 3.7  | 6.6               | 2.1-9.4*   | Czapek   |
|                              | 73.9                      | 70.0              | 7.6  | 4.0-7.0           | 2.1-10.0+*   | Peptone  |
|                              | 100.0                     |                   | 4.1-9.0                                    |                   | 2.5-9.8*   | Beet decoction                                       |
|                              |                           |                   |  |                   |  |  |
| <i>Botrytis cinerea</i>      | 88.8                      |                   | 3.0  |                   | *1.6-6.9   | Mannite  |
|                              | 88.2                      |                   | 3.1  |                   | 2.1-7.9  | Czapek   |
|                              | 92.9                      |                   | 4.0  |                   | 2.1-8.7  | Peptone  |
|                              |                           |                   | 3.0-6.5                                    |                   | 2.0-9.8*   | Beet decoction                                       |
|                              | 68.0                      | 35.3              | 2.5-3.0                                    | 7.2               | 2.1-10.0+*   | Water (HCl or KOH)                                   |
|                              | 18.7                      |                   | 3.0  |                   | 2.0-5.5  | Water (H <sub>2</sub> PO <sub>4</sub> and NaOH)      |
|                              | 95.0                      |                   | 2.4-6.2                                    |                   | *1.6-8.2*  | $\frac{3}{8}$ mannite + $\frac{1}{8}$ beet decoction |
|                              |                           |                   |  |                   |  |  |
|                              | 100.0                     | 96.2              | 3.1  | 6.2-7.3           | 2.1-7.9*   | $\frac{1}{8}$ mannite + $\frac{3}{8}$ beet decoction |
|                              |                           |                   |  |                   |  |  |

TABLE IX (cont.)

| Organisms               | Percentage of germination |                   | Hydrogen-ion concentration, Pa |                   | Media   |
|-------------------------|---------------------------|-------------------|--------------------------------|-------------------|---|
|                         | Primary maximum           | Secondary maximum | Primary optimum                | Secondary optimum |   |
| Fusarium sp.            | 74.1                      | 97.3              | 6.9                            | 3.1               | Mannite   |
|                         | 99.3                      |                   | 8.4                            |                   | Czapek  |
|                         | 99.7                      |                   | 4.0-10.0+                      |                   | Peptone   |
|                         | 99.1                      |                   | 7.0-7.5                        |                   | Beet decoction                                  |
|                         | 66.2                      | 62.4              | 3.0                            | 6.4               | Water (HCl or KOH)                              |
| Penicillium italicum    | 76.5                      | 73.2              | 4.0-5.5                        | 7.7               | Water (H <sub>3</sub> PO <sub>4</sub> and NaOH) |
|                         | 13.1                      | 7.3               | 7.7                            | 3.0-3.6           | Mannite   |
|                         | 46.8                      | 35.8              | 2.6                            | 6.0               | Czapek  |
|                         | 86.2                      |                   | 3.0-4.0                        |                   | Peptone   |
|                         | 67.8                      | 56.0              | 6.8-8.0                        | 3.6-5.0           | Beet decoction                                  |
| Lenzites saepiaria      | 68.0                      |                   | 3.0                            |                   | Mannite   |
|                         | 49.0                      |                   | 3.2                            |                   | Czapek  |
|                         | 92.6                      |                   | 3.0                            |                   | Peptone   |
|                         | 87.6                      | 61.2              | 3.0                            | 7.5               | Beet decoction                                  |
| Puccinia graminis       | 65.1                      |                   | 5.0                            |                   | Mannite   |
|                         | 88.1                      | 86.2              | 3.7                            | 5.9               | Czapek  |
|                         | 84.1                      |                   | 5.5                            |                   | Peptone   |
|                         | 69.8                      | 77.4              | 4.1-5.1                        | 7.0               | Beet decoction                                  |
|                         | 65.4                      |                   | 3.9-5.8                        |                   | Water (HCl or KOH)                              |
| Colletotrichum Gossypii |                           |                   |                                |                   | Mannite   |
|                         |                           |                   |                                |                   | Czapek  |
|                         |                           |                   |                                |                   | Peptone   |
|                         |                           |                   |                                |                   | Beet decoction                                  |

\* Germination obtained in the most acid or in the most alkaline cultures of the experiment.

The relatively high percentages and the extended range of germination as related to hydrogen-ion concentration in a beet

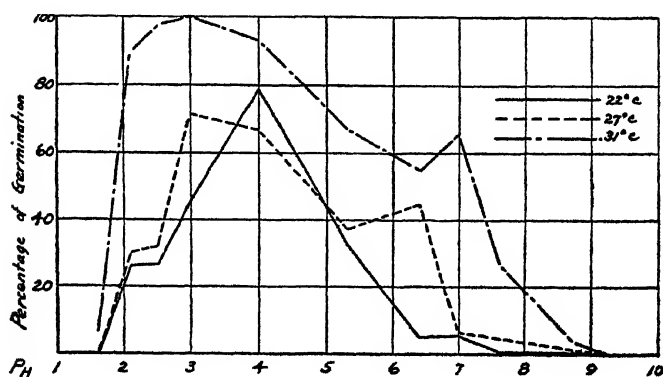


Fig. 9. *Aspergillus niger* in 2 per cent bacto-peptone solution.

decoction medium are very striking, and the extended range of germination in "water HCl or KOH" is equally striking. It would seem, therefore, that either the beet decoction might

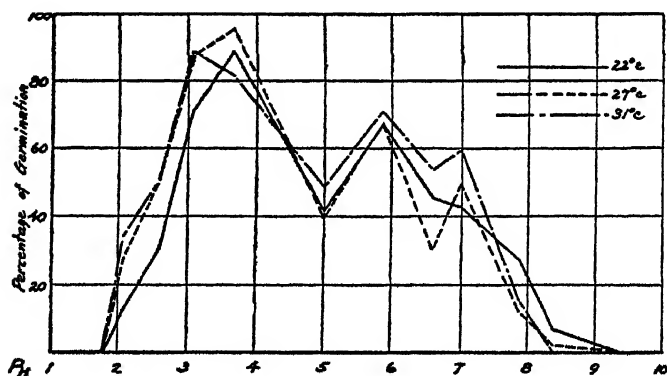


Fig. 10. *Aspergillus niger* in Czapek's full nutrient solution.

possess some stimulating substance, which is absent in the synthetic culture media, or else it might undergo changes in reaction during the process of spore germination. Germination

percentages are relatively low in "water HCl or KOH", but the expansion in range beyond that offered by the other culture solutions—with the exception, of course, of beet decoction—would also tend to indicate a change in reaction during germination. In view of these facts, it seemed advisable to conduct a few simple experiments which might throw light on this problem.

Inasmuch as *A. niger* and *B. cinerea* furnished such widely different results in solutions of mannite and beet decoction, these two organisms were selected for a study of the first aspect mentioned, and the extremely stimulating effect of beet decoction upon germination of these spores is shown by the data accumulated from experiments involving mixtures of the mannite and beet decoction solutions in various proportions, as presented in the tables for the respective organisms.

The same organisms, together with *Fusarium* sp., were employed in studying the second possibility, that is, changes in the reaction of the medium during germination. These data appear in tables X, XI, and XII, and will be considered subsequently in the discussion.

TABLE X

BOTRYTIS CINEREA. INITIAL AND FINAL HYDROGEN-ION CONCENTRATIONS DURING GERMINATION FOR 20 HOURS AT 24° C.

| Hydrogen-ion concentration, $P_H$ |     |     |     |     |     |     |     |     |     |     |     |     | Media   |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---|
| Initial                           | 1.6 | 2.0 | 2.5 | 3.0 | 4.0 | 5.3 | 6.2 | 7.0 | 7.5 | 8.1 | 8.6 | 9.6 | Mannite                                       |
| Final                             | 1.6 | 2.0 | 2.5 | 3.0 | 4.0 | 5.3 | 6.2 | 7.0 | 7.5 | 8.0 | 8.2 | 9.1 |   |
| Control                           | 1.6 | 2.0 | 2.5 | 3.0 | 4.0 | 5.3 | 6.2 | 7.0 | 7.5 | 8.1 | 8.2 | 9.0 |   |
| Initial                           | 1.2 | 1.8 | 2.1 | 2.8 | 3.4 | 3.6 | 5.0 | 6.1 | 6.7 | 7.2 | 8.0 | 8.9 | Czapek  |
| Final                             | 1.2 | 1.8 | 2.1 | 2.8 | 3.4 | 3.6 | 5.0 | 6.1 | 6.7 | 7.2 | 7.8 | 8.2 |   |
| Control                           | 1.2 | 1.8 | 2.1 | 2.8 | 3.4 | 3.6 | 5.0 | 6.1 | 6.7 | 7.2 | 7.8 | 8.2 |   |
| Initial                           | 1.8 | 2.4 | 2.7 | 3.1 | 3.6 | 4.2 | 5.0 | 5.8 | 6.5 | 6.8 | 8.0 | 8.6 | Beet decoction                                |
| Final                             | 1.8 | 2.3 | 3.6 | 3.8 | 3.8 | 4.7 | 4.8 | 5.5 | 5.7 | 6.4 | 6.7 | 7.9 |   |
| Control                           | 1.8 | 2.4 | 2.7 | 3.2 | 3.8 | 4.4 | 5.0 | 5.8 | 6.6 | 7.0 | 8.1 | 8.3 |   |
| Initial                           | 1.6 | 2.0 | 2.4 | 3.0 | 4.0 | 5.5 | 6.2 | 7.0 | 7.7 | 8.1 | 8.2 | 8.6 | Water (H <sub>2</sub> PO <sub>4</sub> & NaOH) |
| Final                             | 1.6 | 2.0 | 2.4 | 3.0 | 4.1 | 5.5 | 6.2 | 7.0 | 7.6 | 8.1 | 8.2 | 8.6 |   |
| Control                           | 1.6 | 2.0 | 2.4 | 3.0 | 4.1 | 5.5 | 6.2 | 7.0 | 7.7 | 8.1 | 8.2 | 8.6 |   |
| Initial                           | 1.4 | 2.0 | 2.4 | 2.9 | 4.0 | 5.1 | 5.5 | 6.3 | 6.6 | 6.9 | 6.9 | 7.1 | Water (HCl or KOH)                            |
| Final                             | 1.4 | 2.0 | 2.4 | 3.2 | 6.0 | 6.7 | 6.8 | 6.7 | 6.9 | 7.1 | 7.1 | 7.1 |   |
| Control                           | 1.4 | 2.0 | 2.4 | 2.9 | 4.6 | 6.2 | 6.6 | 6.7 | 6.9 | 7.1 | 7.2 | 7.3 |   |



TABLE XI

ASPERGILLUS NIGER. INITIAL AND FINAL HYDROGEN-ION CONCENTRATIONS DURING GERMINATION FOR 20 HOURS AT 24° C.

| Hydrogen-ion concentration, $P_H$ |     |     |     |     |     |     |     |     |     |     |     |     |     | Media               |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------------------|
| Initial                           | 1.6 | 2.0 | 2.5 | 3.0 | 4.0 | 5.3 | 6.2 | 7.0 | 7.5 | 8.1 | 8.6 | 9.6 |     | Mannite             |
| Final                             | 1.6 | 2.1 | 2.6 | 3.1 | 4.0 | 5.4 | 6.3 | 7.0 | 7.1 | 8.1 | 8.2 | 9.0 |     |                     |
| Control                           | 1.6 | 2.0 | 2.5 | 3.0 | 4.0 | 5.3 | 6.2 | 7.0 | 7.5 | 8.1 | 8.2 | 9.0 |     |                     |
| Initial                           | 1.2 | 1.8 | 2.1 | 2.8 | 3.4 | 3.6 | 5.0 | 6.1 | 6.7 | 7.2 | 8.0 | 8.9 | 9.6 | Czapek              |
| Final                             | 1.2 | 1.8 | 2.1 | 2.8 | 3.5 | 3.6 | 5.2 | 6.2 | 6.8 | 7.2 | 7.8 | 8.1 | 8.9 |                     |
| Control                           | 1.2 | 1.8 | 2.1 | 2.8 | 3.4 | 3.6 | 5.0 | 6.1 | 6.7 | 7.2 | 7.8 | 8.2 | 9.0 |                     |
| Initial                           | 1.8 | 2.4 | 2.7 | 3.1 | 3.6 | 4.2 | 5.0 | 5.8 | 6.5 | 6.8 | 8.0 | 8.6 | 8.9 | Beet decoc-<br>tion |
| Final                             | 1.8 | 2.4 | 2.8 | 3.7 | 3.9 | 4.5 | 4.9 | 5.5 | 5.7 | 5.9 | 6.4 | 6.4 | 7.4 |                     |
| Control                           | 1.8 | 2.4 | 2.7 | 3.2 | 3.8 | 4.4 | 5.0 | 5.8 | 6.6 | 7.0 | 8.1 | 8.3 | 8.8 |                     |

TABLE XII

FUSARIUM SP. INITIAL AND FINAL HYDROGEN-ION CONCENTRATIONS DURING GERMINATION FOR 20 HOURS AT 27° C.

| Hydrogen-ion concentration, $P_H$ |     |     |     |     |       |       |       |     |     |     |     |     |     | Media                           |
|-----------------------------------|-----|-----|-----|-----|-------|-------|-------|-----|-----|-----|-----|-----|-----|---------------------------------|
| Initial                           | 1.6 | 2.0 | 2.4 | 3.0 | 4.0   | 5.3   | 6.2   | 7.0 | 7.6 | 8.0 | 8.2 | 8.8 | 9.6 | Mannite                         |
| Final                             | 1.6 | 2.0 | 2.4 | 3.0 | 4.0   | 5.3   | 6.2   | 7.0 | 7.5 | 7.9 | 8.1 | 8.5 | 9.0 |                                 |
| Control                           | 1.6 | 2.0 | 2.4 | 3.0 | 4.0   | 5.3   | 6.2   | 7.0 | 7.6 | 8.0 | 8.2 | 8.5 | 9.0 |                                 |
| Initial                           | 1.2 | 1.8 | 2.1 | 2.8 | 3.4   | 3.6   | 5.0   | 6.1 | 6.7 | 7.2 | 8.0 | 8.9 | 9.6 | Czapek                          |
| Final                             | 1.2 | 1.8 | 2.1 | 2.8 | 3.5   | 4.2   | 5.6   | 6.2 | 6.7 | 7.2 | 7.6 | 8.1 | 8.8 |                                 |
| Control                           | 1.2 | 1.8 | 2.1 | 2.8 | 3.4   | 3.6   | 5.0   | 6.1 | 6.7 | 7.2 | 7.8 | 8.2 | 9.0 |                                 |
| Initial                           | 1.5 | 2.0 | 2.4 | 2.9 | 4.0   | 5.5   | 6.2   | 7.0 | 7.7 | 8.1 | 8.3 | 8.6 |     | Water<br>( $H_2PO_4$<br>& NaOH) |
| Final                             | 1.5 | 2.0 | 2.4 | 2.9 | (4.4) | (5.6) | (6.3) | 7.0 | 7.7 | 8.2 | 8.3 | 8.7 |     |                                 |
| Control                           | 1.5 | 2.0 | 2.4 | 3.0 | 4.6   | 5.5   | 6.2   | 7.0 | 7.7 | 8.1 | 8.3 | 8.7 |     |                                 |
| Initial                           | 1.4 | 2.0 | 2.4 | 2.9 | 4.0   | 5.1   | 5.5   | 6.3 | 6.6 | 6.9 | 6.9 | 7.1 | 8.6 | Water<br>(HCl or<br>KOH)        |
| Final                             | 1.4 | 2.1 | 2.5 | 5.5 | 6.7   | 6.9   | 7.2   | 7.1 | 7.2 | 7.2 | 7.3 | 7.5 | 7.7 |                                 |
| Control                           | 1.4 | 2.0 | 2.4 | 2.9 | 4.6   | 6.2   | 6.6   | 6.7 | 6.9 | 7.1 | 7.2 | 7.3 | 7.7 |                                 |

## DISCUSSION

Germination of fungous spores is a subject that has engaged the attention of botanists—physiologists and pathologists—for many years. Various aspects of the problem have been extensively studied, and certain factors, such as temperature, light, moisture, and reaction of the medium have been experimentally considered. The toxic properties of H and OH ions have been treated more or less, but hydrogen-ion concentration has so recently become biologically important that the influence of this factor in germination was practically new. Having no technique they could apply for the direct determination of hydrogen-ion concentration, the earlier investigators, like many of the later ones, frequently employed conductivity data in making their interpretations. This method is, however, inapplicable when other solutes are introduced, and the presence of strong buffers, whether inorganic or organic, would render most difficult any computation of active acidity or alkalinity.

It is believed that the results here presented are sufficient

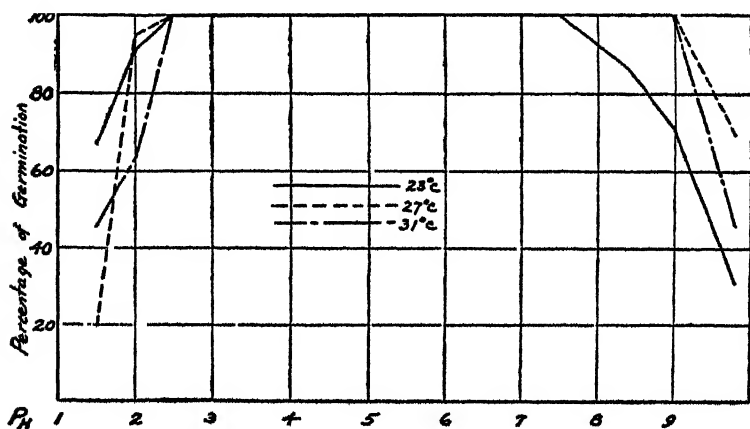


Fig. 11. *Aspergillus niger* in sugar beet decoction.

to change materially the prevailing view as to the relation of spore germination to acid and alkaline media. Although germination, as related to active acidity and alkalinity, varies with the organism and with the liquid culture medium, it is a process

which is strikingly supported by a relatively high H-ion concentration. In various forms, notably in *Penicillium* and *Fusarium*, and under certain conditions, secondary maxima at or near the neutral point occur, and these often approach in magnitude the primary maxima on the acid side. It is not necessary, of course, to assume that the H-ion concentration most favorable for germination will also prove most favorable for the continued growth and development of the organism. In this work, it was found that germinating spores of *P. graminis* conspicuously exhibit only rudimentary germ tubes or knob-like projections

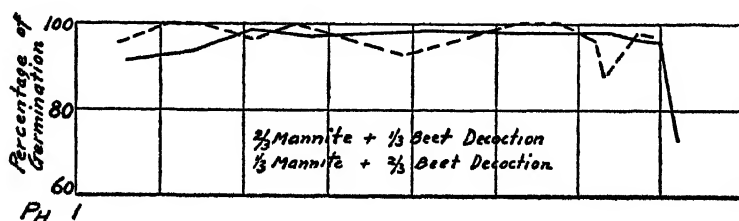


Fig. 12. *Aspergillus niger* in a medium composed of M/5 mannite and sugar beet decoction (25°C.).

————— 66 2/3 per cent mannite + 33 1/3 per cent sugar beet decoction.

----- 33 1/3 per cent mannite + 66 2/3 per cent sugar beet decoction.

in the most alkaline nutrient cultures included within the favorable range and that all of the fungi show abnormal and irregularly-shaped germ tubes in the most acid cultures allowing germination, indicating, therefore, that such reactions while favorable for germination are unfavorable for growth. The germ tubes of *B. cinerea* disintegrate at  $P_H$  2.1.

Nutrient media for pathological and bacteriological work are usually neutral or slightly acid, while my data show that increasing concentrations of hydrogen ions from neutrality to approximately  $P_H$  3.0–4.0, in the various culture media, favorably influence the germination of the spores of *B. cinerea*, *A. niger*, *P. cyclopium*, *P. italicum*, *P. graminis*, *L. saepiaria*, and *Fusarium* sp. However, with increase of hydrogen-ion concentration above this zone, the germination quantities abruptly diminish and soon reach a zero value. Under conditions of moderate active

alkalinity, on the other hand, relatively low percentages of germination are obtained in most cases. Some detailed discussion is needed in order to compare adequately these results and at the same time to consider the work of others.



Fig. 13. *Penicillium cyclopium* in M/5 mannite solution.

From my results, *B. cinerea* may be regarded either as extremely sensitive to active alkalinity in solutions of mannite, Czapek,

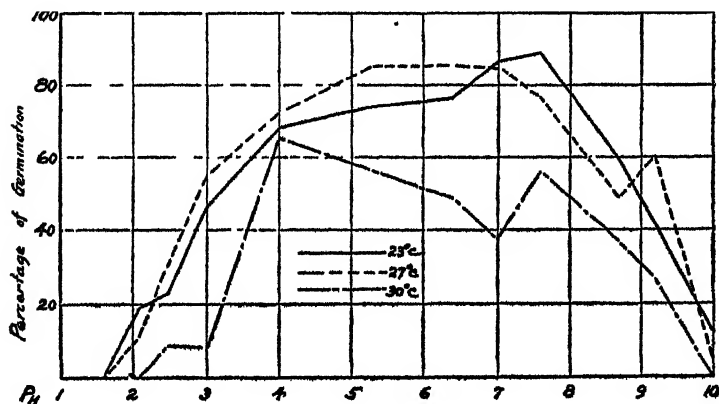


Fig. 14. *Penicillium cyclopium* in 2 per cent bacto-peptone solution.

peptone, and "water  $H_3PO_4$  and  $NaOH$ " and to a less degree in beet decoction and "water  $HCl$  or  $KOH$ ," or else as manifest-

ing a certain dependence upon the stimulating effects of hydrogen ion concentration in such media. Maximum germination generally occurs between  $P_H$  2.5 and 4.0, but in the case of beet decoction the optimum zone extends from  $P_H$  3.0 to 6.5. A secondary maximum near neutral is shown only in the case of "water HCl or KOH." The favorable range on the acid side extends more or less uniformly to  $P_H$  2.0 in the solutions, but varies considerably in the alkaline solutions. The narrowest range of germination is exhibited in "water HCl or KOH,"

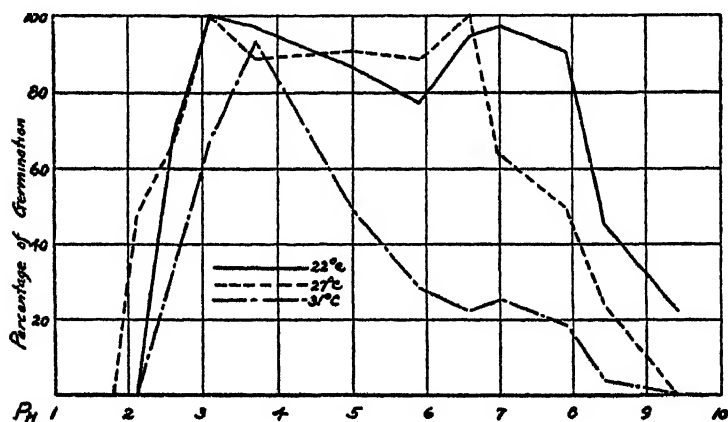


Fig. 15. *Penicillium cyclopium* in Czapek's full nutrient solution.

extending to  $P_H$  5.5, and followed in order by solutions of mannite,  $P_H$  6.9, Czapek,  $P_H$  7.9, peptone,  $P_H$  8.7, beet decoction,  $P_H$  9.8, and "water HCl or KOH,"  $P_H$  10.0 +. The results obtained with these spores in solutions of mannite are very similar to those previously presented by the writer. It may be well to cite the concentrations of certain acids and alkalis allowing normal or almost normal development of the spores of *Botrytis vulgaris* in beet decoction, as determined by Clark ('99): HCl, N/128;  $HNO_3$ , N/256;  $H_2SO_4$ , N/64; acetic acid, N/256; monochloroacetic acid, N/512; dichloroacetic acid, N/256; trichloroacetic acid, N/128; HCN, N/8192; KOH, N/64; and  $NH_4OH$ , N/256. He concludes that the hydroxyl group, OH, is rather more toxic to the moulds studied than ionic H.

*A. niger* exhibits a relation to hydrogen-ion concentration similar to that of *B. cinerea*. Maximum germination is furnished

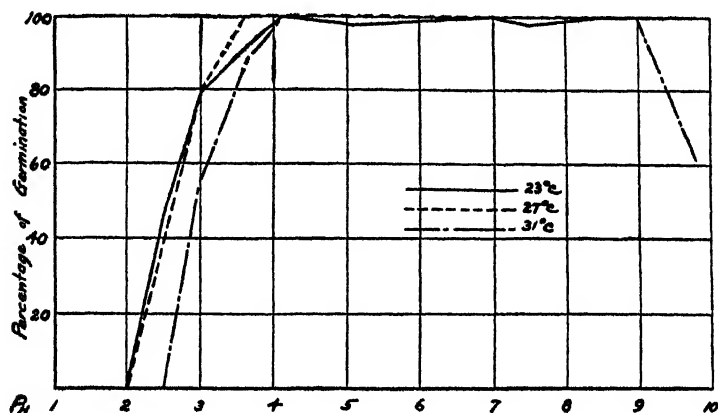


Fig. 16. *Penicillium cyclopium* in sugar beet decoction.

between  $P_H$  3.0 and 4.0 in all media except beet decoction. Here the range extends from the mentioned concentration to  $P_H$  7.5. Low percentages of germination, except in beet decoction, occur under conditions of active alkalinity. Clark found

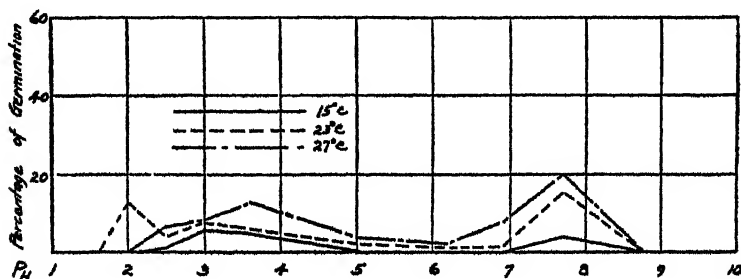


Fig. 17. *Penicillium italicum* in M/5 mannite solution.

that the spores of *Aspergillus flavus* were somewhat more resistant to the various acids and alkalis in beet decoction than were those of "*Botrytis vulgaris*." The results presented in this paper for *A. niger* and *B. cinerea* further substantiate this relation, and the data obtained for the former in solutions of

mannite are consistent with those previously published by the writer.

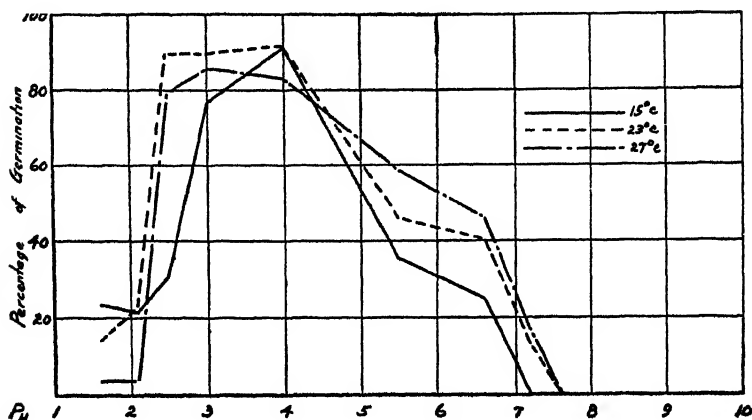


Fig. 18. *Penicillium italicum* in 2 per cent bacto-peptone solution.

The spores of *P. cyclopium* germinated under conditions very similar to those described for *B. cinerea* and *A. niger*, but a tendency is manifested by these spores to germinate more freely

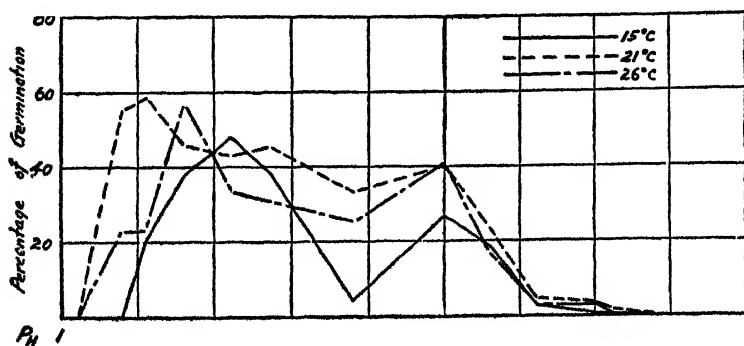


Fig. 19. *Penicillium italicum* in Czapek's full nutrient solution.

under neutral and slightly alkaline conditions. Two maxima, one from  $P_H$  3.0 to 4.0 and the other about  $P_H$  7.0, frequently appear. Of all the forms which he studied, Clark found *Penicillium glaucum* the most resistant to acids and alkalis, as well

as to other poisons, the inhibiting concentrations on the whole being greater than those submitted for *B. vulgaris*. Stevens' results indicated that *Penicillium crustaceum* is more resistant to poisons in aqueous solutions than any of the other fungi studied by him. Growth occurred in N/50 HCl and H<sub>2</sub>SO<sub>4</sub>,

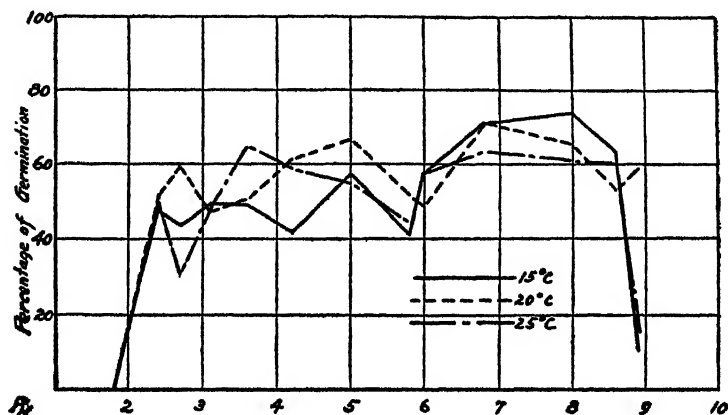


Fig. 20. *Penicillium italicum* in sugar beet decoction.

while N/40 KOH and NaOH caused death. While the germination curves for *P. cyclopium* previously obtained in solutions of mannite do not agree in every detail with the curves reported in this paper for the same medium, certain features are similar.

An acid reaction decidedly favors the growth of *Penicillium italicum* and under certain conditions germination is here shown to be influenced by similar conditions. The highest germination in the peptone and in the Czapek's solutions is between P<sub>H</sub> 2.0 and 4.0 and practically no germination occurs beyond the neutral point. The data further show that germination takes place more quickly in the peptone medium than in any other, this being the only instance in which germination takes place readily after an incubation interval of 10 hours. A period of 20 hours is required in all other cases. Beet decoction, on the other hand, affords best germination in the alkaline solutions, but the difference between the highest average on the acid side and that on the alkaline side is very slight. The germ tubes in the alkaline cultures are short and stubby and give few signs of growth



and development. Growth and development in the acid cultures, however, are decidedly greater. This recalls the question

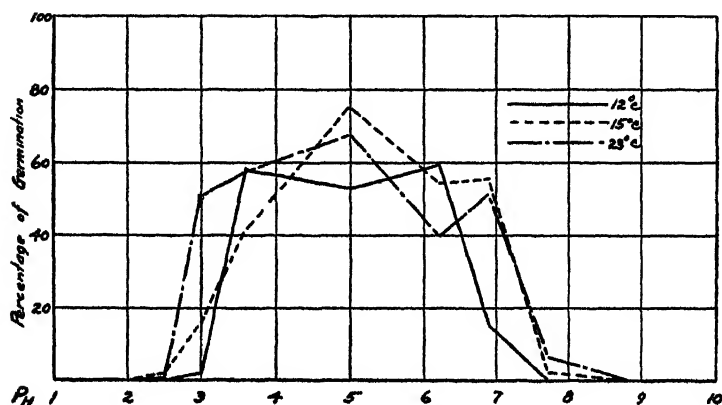


Fig. 21. *Puccinia graminis* in M/5 mannite solution.

suggested by Duggar ('01), of media stimulating germination and not growth, and vice versa. Maximum germination, as determined from my experiments, occurs within zones rather

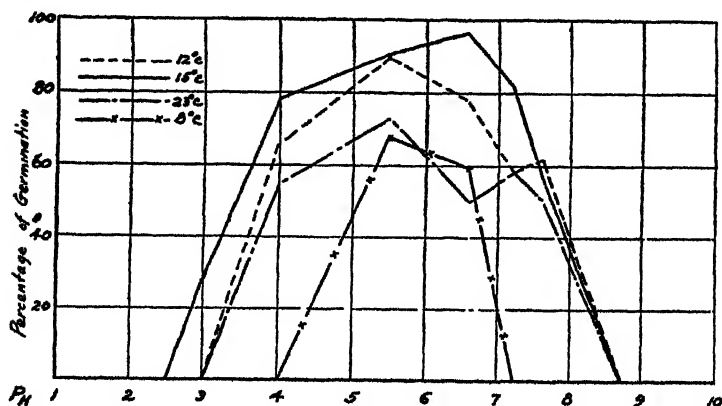


Fig. 22. *Puccinia graminis* in 2 per cent bacto-peptone solution.

than at definite points.

*L. saepiaria*, like certain other species included within the acid group, proved very sensitive to an alkaline reaction, and while the limiting concentration on the acid side is somewhat lower

than in the case of other included forms, these fungi are more or less similar in behavior. The results obtained in the various culture solutions are consistent and comparable. Certain stimulating effects, it will be noted, are exhibited by the neutral cultures of the beet decoction, but the favorable range in this medium does not expand as it does for certain of the other fungi. Meacham ('18) obtained inhibition of growth of *L. saepiaria* at approximately  $P_H$  1.7 in synthetic media and malt-extract, and it is of interest to note that he frequently obtained

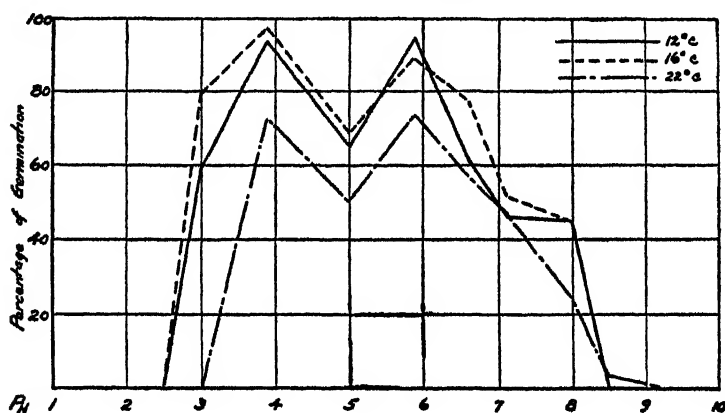


Fig. 23. *Puccinia graminis* in Czapek's full nutrient solution.

a maximum of growth at about  $P_H$  3.0, which approaches very closely the hydrogen-ion concentration in the mannite, Czapek, peptone, and beet decoction solutions which afford maximum germination.

The uredospores of *Puccinia graminis* do not germinate, on the whole, in solutions with as high hydrogen-ion concentrations as some of the other fungi employed, the favorable range terminating between  $P_H$  2.5 and 3.0 as compared with  $P_H$  1.5 to 2.0 in other cases. Relatively low percentages of germination are obtained under conditions of active alkalinity, except in the case of "water HCl or KOH," and such percentages vary materially with the medium. In "water HCl or KOH," germination occurs freely at  $P_H$  9.4, a hydrogen-ion concentration producing inhibition of germination with the other media.

The expansion of favorable range on the alkaline side in water is striking and analogous to what resulted with *Botrytis*. A

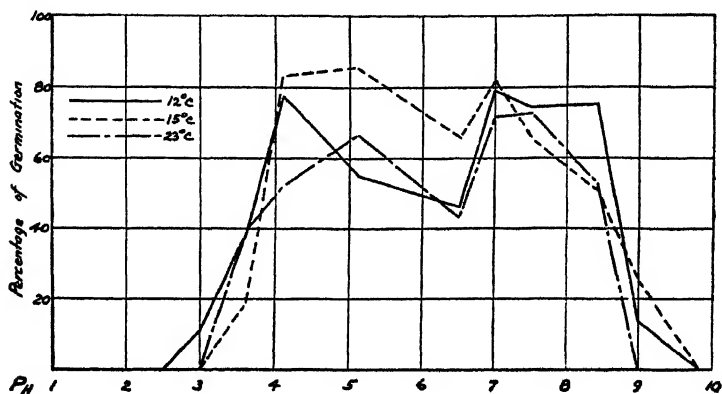


Fig. 24. *Puccinia graminis* in sugar beet decoction.

slightly acid or neutral reaction, on the whole, decidedly stimulates the germination of these spores.

Of the forms studied, *Fusarium* sp. is the only one that de-

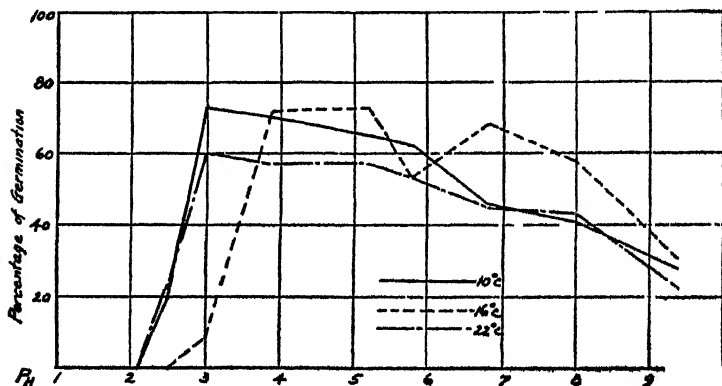


Fig. 25. *Puccinia graminis* in "water HCl or KOH."

cidedly responds to an alkaline medium. This form, moreover, exhibits the widest range of germination with respect to the reaction of the medium, extending from  $P_H$  2.0 to  $P_H$  10.0+, the most alkaline culture. Maxima generally appear about  $P_H$  3.0-4.0 and near  $P_H$  7.0.

The spores of *C. Gossypii* are very variable in germination and give no definite and characteristic curve of germination.

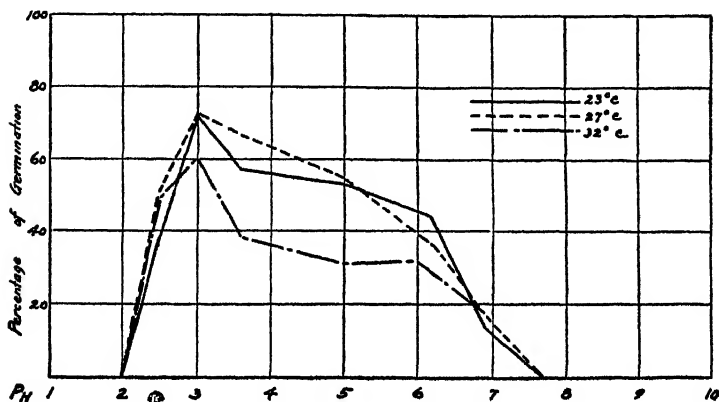


Fig. 26. *Lenzites saepiarum* in 1/5 mannite solution.

Beyond emphasizing the favorable effects of an alkaline or slightly acid medium, nothing can be said. In the case of the peptone solutions, the usual type of germination did not occur;

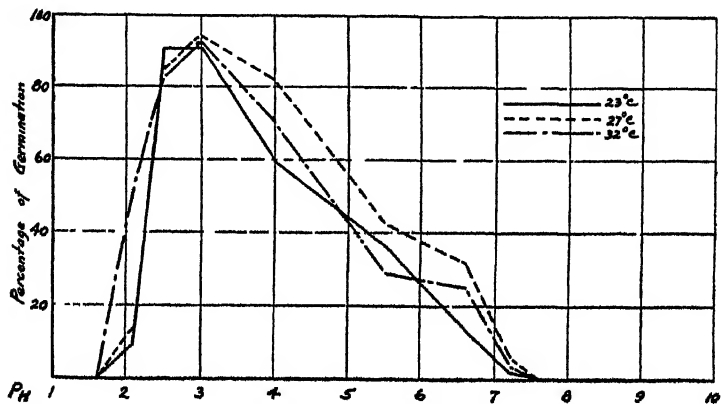


Fig. 27. *Lenzites saepiarum* in 2 per cent bacto-peptone solution.

that is, not with the production of germ tubes. On the contrary, secondary spores were formed at the ends of the mature spores, and the data in peptone solution for this organism represent percentage quantities of this phenomenon. The remaining

culture solutions, on the other hand, gave normal germination. For 10 hours at 23° and 27° C., the secondary spores were still attached; at 31° C., however, they were detached. When incubated for 20 hours at each of the temperatures, the spores were detached and numerous. According to Stoneman ('98), "the formation of the so-called secondary spores or buds which are common to *Gloeosporium*, *Colletotrichum*, *Volutella*, and *Vermicularia* is not a constant character, but may be absent throughout the entire cycle of development of a species, or may be forced in the same species by a lack of nourishment."

The spores of *Ustilago Avenae* from the material available gave such relatively low percentages of germination with the

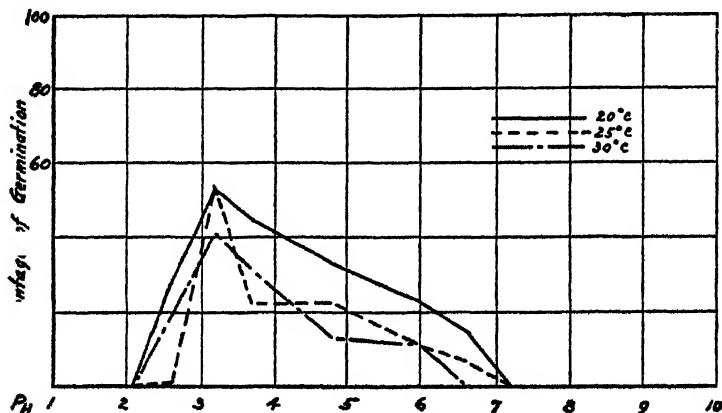


Fig. 28. *Lenzites saepeparia* in Czapek's full nutrient solution.

described method of technique that this organism was discarded. However, series were conducted in mannite solutions at 15°, 19°, and 25° C. In general, germination ranges from  $P_H$  2.4 to 8.2, with a maximum at  $P_H$  6.2. Sporidia occur most abundantly from  $P_H$  5.4 to 7.0 and decrease in number with increase in departure from either side of this zone.

Repeated endeavors have also been made to study conidial germination with certain of the powdery mildews, but all attempts have been without avail. No germination whatever has thus far been obtained with *Sphaerotheca pannosa* at different temperatures when the conidia were placed either in the hanging

drop or in film cultures of solutions of mannite, sucrose, beet decoction, tap water, and distilled water. Previous subjection of the spores for various time intervals to low or freezing temperatures also failed to stimulate germination.

Marked differences in requirements for germination are shown by the spores of fungi. Some are capable of germinating in moist air or in water, while others are capable of germinating only in the presence of a nutrient solution or special stimulus. The percentages of germination, in many cases, depend largely

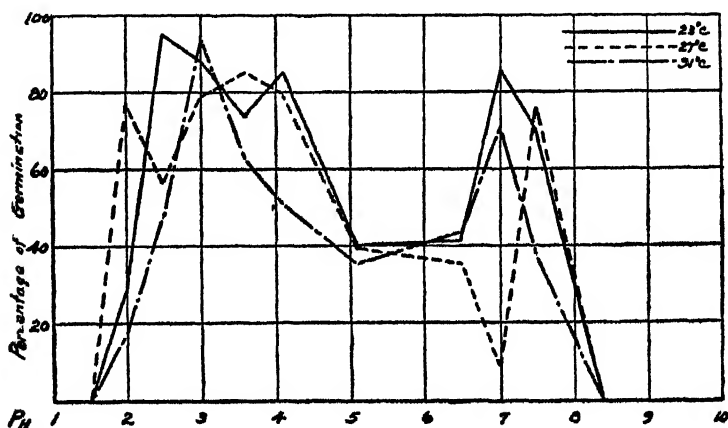


Fig. 29. *Lenzites saepiaria* in sugar beet decoction.

upon the direct food value of the medium; that is, the most perfect food affords the best germination. Certain of the fungi, however, germinate well in sugar solutions and best in plant infusions or decoctions. Such cases, then, may be classed as food stimuli. Organic acids are generally regarded as feeble stimuli for the germination and growth of fungi, but here again the matter is vitally associated with the question of toxicity. Taylor ('17) determined the concentrations of a few organic and inorganic acids necessary to check the growth of various organisms. His data led him to conclude that there is a great variation in specificity in the relation of such acids to different organisms.

Brooks ('06) found that the deleterious action of  $\text{CuSO}_4$ ,  $\text{HNO}_3$ , and  $\text{H}_2\text{SO}_4$  in beet decoction medium upon the germination and development of certain fungous spores was least at a provisional optimum temperature and that it increased very rapidly with rise in temperature above such an optimum. The effects of the three chemicals, however, were very different, and spores inhibited by cold were not greatly injured when exposed to harmful agents.

Temperature variations such as were employed in the experiments here reported exert little or no influence upon the curves

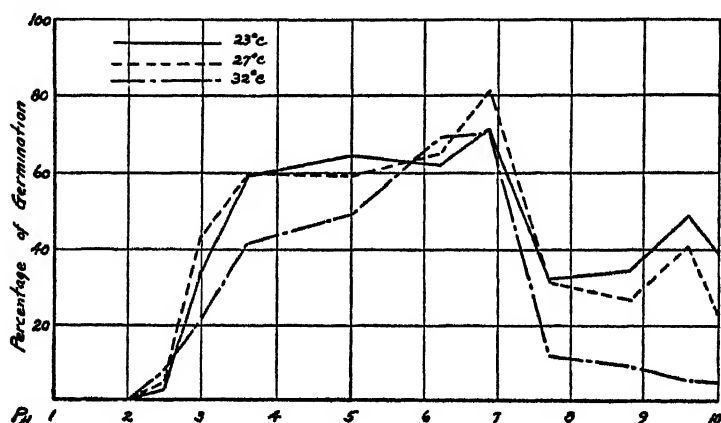


Fig. 30. *Fusarium* sp. in M/5 mannite solution

of germination, but it must be remembered that the temperature range was relatively narrow. This aspect of the problem will be considered later.

The effect of the hydrogen-ion concentration of the medium as influenced by certain types of nutrition, or the chemical composition of the nutrient medium, is shown by the results presented in this paper to be of extreme importance. Inasmuch as a detailed consideration has been devoted to these points for the individual fungi, it is necessary to mention only a few of the striking features. The results with the various media are comparable, yet it is difficult to make general statements that would be applicable in all cases.

"Water  $\text{H}_3\text{PO}_4$  and  $\text{NaOH}$ " followed by solutions of mannite, undoubtedly gives the lowest percentages and the smallest range of germination. The conditions in the two solutions are very comparable; that is, the individual cultures contain equal quantities of  $\text{H}_3\text{PO}_4$  and increasing quantities of  $\text{NaOH}$ , resulting therefore in the formation of  $\text{NaH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$  at  $P_H$  4.5 and  $P_H$  9.2 respectively. No  $\text{Na}$  ions are contained in the most acid culture and these ions increase in number with decrease in  $\text{H}$ -ion concentration. It is impossible, however, to

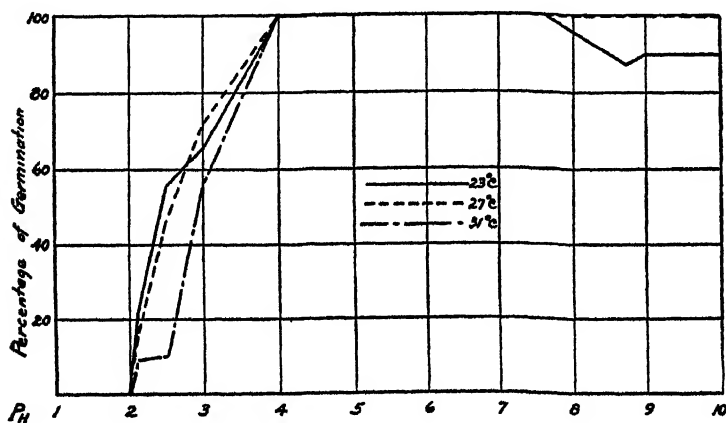


Fig. 31. *Fusarium* sp. in 2 per cent bacto-peptone solution.

vary the  $\text{H}$ -ion concentration without varying the concentration of some other ion or ions at the same time, even though such variations may be insignificant.

Solutions of bacto-peptone give very similar results to solutions of mannite, except that germination is better, that the range is slightly more extended on the alkaline side, and that maximum germination frequently occurs in zones rather than at definite points. The reaction of the peptone solutions, as well as Czapek's, beet decoction, and "water  $\text{HCl}$  or  $\text{KOH}$ ," is varied on the acid side by additions of  $\text{HCl}$  and on the alkaline side by additions of  $\text{KOH}$ . Disregarding the variation in  $\text{H}$ -ion concentration, the acid cultures thus contain increasing amounts of  $\text{Cl}$  ions and the alkaline cultures increasing amounts of  $\text{K}$  ions.



Czapek's full nutrient solution generally exhibits germination ranges similar to those afforded by the peptone solutions, but the curves of germination in the Czapek differ decidedly from those developed in the peptone. Invariably two maxima occur: one between  $P_H$  3.0 and 4.0 and the other between  $P_H$  6.0 and 7.0, and these maxima present more or less definite peaks in the curve. It is interesting to note that there is frequently a decided decrease of germination quantities at or near  $P_H$  5.0. This minimum within the favorable range occurs in the culture containing the normal Czapek full nutrient solution without additions of either acid or alkali.

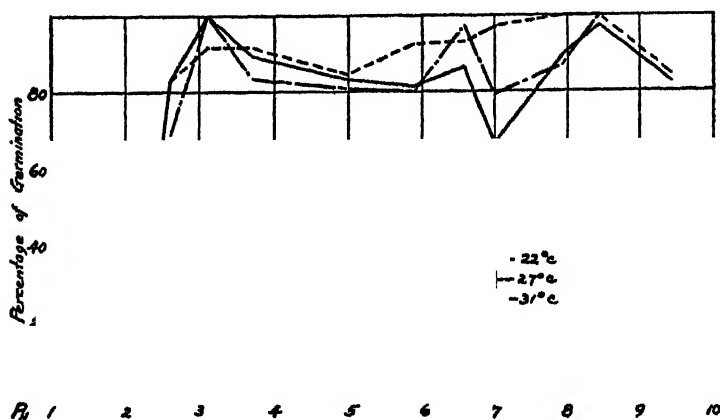


Fig. 32. *Fusarium* sp. in Czapek's full nutrient solution.

"Water HCl or KOH" allows very wide ranges in germination as well as maxima on the acid and alkaline sides of neutrality. The spores of *Botrytis cinerea* and *Puccinia graminis* germinate freely in aqueous solutions with hydrogen-ion concentrations which in most of the other culture solutions would have caused inhibition of germination.

Beet decoction, it has been shown, furnishes highest percentages and the widest range of germination as related to hydrogen-ion concentration for all of the spores employed in this investigation. Germination occurs freely under conditions of active alkalinity in this medium, and hydrogen-ion concentrations

producing inhibition of germination in the other media allow good germination in this medium. The maxima generally

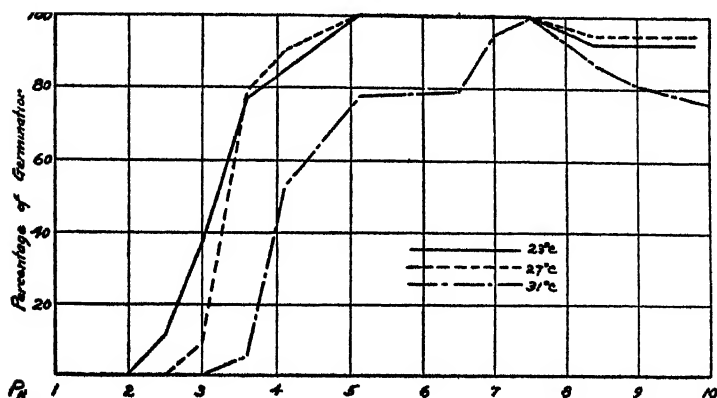


Fig. 33. *Fusarium* sp. in sugar beet decoction.

manifest themselves within relatively wide zones, and perfect or almost perfect germination frequently continues from extreme acidity to extreme alkalinity, that is, within the limits of the

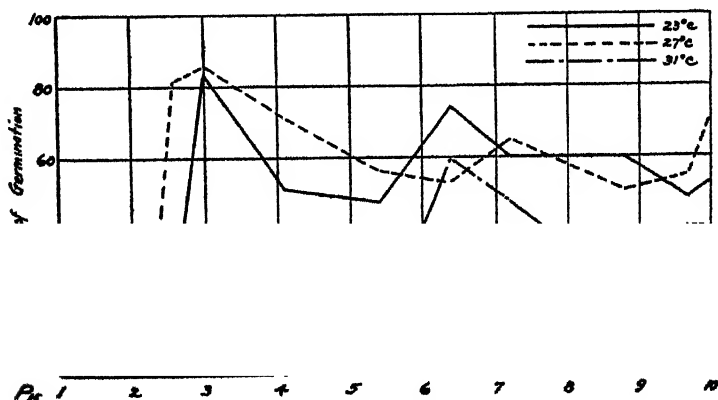


Fig. 34. *Fusarium* sp. in "water HCl or KOH."

experiments. From these facts, it would seem that the beet decoction possesses some stimulating substance or complex set of conditions, either or both of which are absent in the synthetic culture media, and the belief is further confirmed by several

experiments reported in this paper. Various additions of beet decoction to solutions of mannite invariably allow stimulation of germination with *A. niger* and *B. cinerea*. In both cases the germination quantities and the range of favorable germination approach those offered by the beet decoction alone and practically no resemblance to those furnished by the mannite solutions is recognizable. In the case of *B. cinerea*, the degree of stimulation near the alkaline extreme varies directly with the quantity



Fig. 35. *Fusarium* sp. in "water  $H_3PO_4$  and  $NaOH$ " ( $25^\circ C.$ ).

of added beet decoction, but no such relation seems to exist in the case of *A. niger*. Duggar ('01) reports a similar stimulation of certain fungous spores in plant decoctions or infusions. He obtained various small percentages of germination of *Coprinus fimetarius* in different vegetable decoctions, but otherwise no germination. *Coprinus micaceus* gave little or no germination in all solutions containing no plant decoction, but furnished perfect germination in bean and dung decoctions. Since plant decoctions are such excellent growth media, it would seem that the stimulus to germination would be a food stimulus. Duggar designed and conducted experiments to determine the stimulus in such cases, and, while this most complicated matter is still unsolved, his statement is as applicable to-day as it was originally, namely: "If the stimulus is that of food, it must be considered in the class of peculiar foods."

The titration curves of the liquid culture media, fig. 1, show that "water HCl or KOH" has the least buffer action and that beet decoction has very little more. These facts, then, would seem to offer an explanation of the expanded favorable range under conditions of active alkalinity, with such solutions on the basis of buffer effect or change in reaction of the medium during germination. In this connection, Itano and Neil ('19) have recently shown that the spores of *Bacillus subtilis* germinate

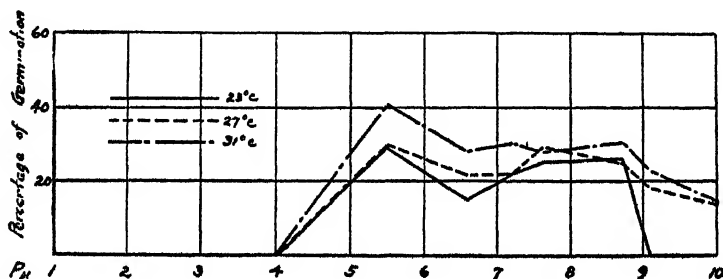


Fig. 36. *Colletotrichum Gossypii* in 2 per cent hacto-peptone solution.

in a broth medium between  $P_H$  5.0 and 10.0, and that few spores ordinarily germinate at the H-ion concentrations closely approaching those of inhibition. In a few days, however, the spores germinate and exhibit all characteristic phenomena, thus indicating that by the life processes of the organism the reaction of the medium approaches that of the optimum. The final hydrogen-ion concentration of the medium, as determined from numerous other contributions for the bacteria, depend upon (1) the organism, (2) the composition of the medium, (3) the initial reaction, (4) the buffer effect, (5) the nature of the acid, (6) the period of incubation, and (7) other conditions favoring or hindering growth. It must be emphasized here that the  $P_H$  values which appear in this paper always represent, unless otherwise specified, the initial reaction or hydrogen-ion concentration of the medium.

The results of my experiments dealing with this phase of the problem do not seem to warrant an explanation entirely on the basis of change reaction of the medium during germination. The matter appears to be further complicated and obscured by

other factors. *A. niger* produces no changes in solutions of mannite or in Czapek's solution, but does cause certain shifts towards neutrality in the alkaline cultures of the beet decoction. This organism produces in the presence of sugar an extremely large quantity of acid during growth, and it is not surprising

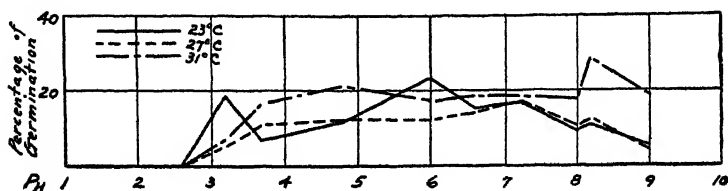


Fig. 37. *Colletotrichum Gossypii* in Czapek's full nutrient solution.

that such reversions do occur in beet decoction, a solution poorly buffered and containing a high percentage of sugar. *B. cinerea* produces no changes in reaction while germinating in solutions of mannite, Czapek's solution, "water  $H_3PO_4$  or  $NaOH$ " and "water  $HCl$  or  $KOH$ ." However, it does produce changes in the beet decoction solutions similar to, but in less degree than

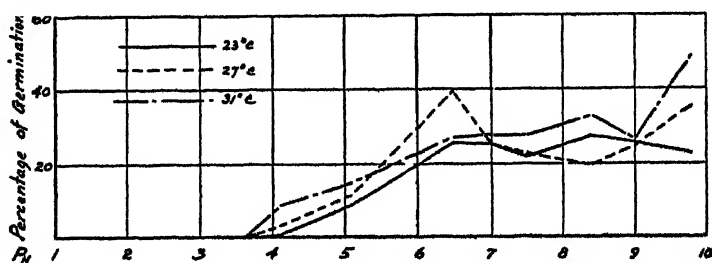


Fig. 38. *Colletotrichum Gossypii* in sugar beet decoction.

*A. niger*. The fact that no change whatsoever in reaction occurs with water lacking buffer effect and that relatively slight changes occur in beet decoction, a medium also poorly buffered, would seem to eliminate the possibility of explaining germination under conditions of active alkalinity entirely on the basis of change in reaction during this process. *Fusarium* sp. causes no changes in reaction of mannite and "water  $H_3PO_4$  or  $NaOH$ ", during



certain minor differences exist, but considering the great variability of spores, varying percentages of perfect germination, regardless of all precautions observed, will undoubtedly occur in any medium which is not a strong stimulus for germination. Germination at H-ion concentrations approaching more or less closely those causing inhibition is frequently irregular, and, in such cases the range of germination is usually widest at the provisional optimum.

The question of hydrogen-ion concentration relations will no doubt assist in the explanation of such problems in parasitism as host resistance, the establishment of strains of the parasite, etc.; and the varied capacity for germination of fungous spores may be found in time to be a suitable criterion for the isolation of strains. Nevertheless, the facts indicate that it is unwise to say that certain species of fungi or certain strains of fungi always germinate exclusively within a definite range of hydrogen-ion concentration. The limiting hydrogen-ion concentration of a fungus, as determined by germination, should be defined in terms of composition of the medium, and unless this is done—together with a consideration of other related factors—no classification of organisms can be made entirely upon any such basis.

#### SUMMARY

Using spores of the eight organisms, *Botrytis cinerea*, *Aspergillus niger*, *Penicillium cyclopium*, *P. italicum*, *Lenzites saepiaria*, *Puccinia graminis*, *Fusarium* sp., and *Colletotrichum Gossypii*, a comparative study has been made dealing with the effects of the hydrogen and hydroxyl-ion concentration upon germination in solutions of mannite, peptone, Czapek's full nutrient, sugar beet decoction, "water  $H_2PO_4$  and  $NaOH$ " and "water  $HCl$  or  $KOH$ ." Under the conditions described and as far as the experiments have gone, the following features of particular interest may be enumerated:

- (1) Germination is a process which is strikingly supported by conditions of active acidity; relatively low percentages of germination, in most cases, are obtained under conditions of active alkalinity; and nutrition exerts a pronounced influence upon the relations of spore germination to the reaction of the medium.

(2) Successively increasing concentrations of hydrogen ions in the various culture solutions, from neutrality to approximately  $P_H$  3.0 or 4.0, influence favorably the germination of the spores of *B. cinerea*, *A. niger*, *Penicillium cyclopium*, *P. italicum*, *Puccinia graminis*, *L. saepiaria*, and *Fusarium* sp. Moreover, *Fusarium* sp. germinates equally as well, if not better, with an alkaline reaction, and *C. Gossypii* is the only species which favorably responds only to an alkaline reaction.

(3) The majority of the fungi employed exhibit a distinct maximum of germination between  $P_H$  3.0 and 4.0. Certain forms, notably *Fusarium* sp., exhibit secondary maximum at or near  $P_H$  7.0.

(4) The range of germination and the magnitude of the germination quantities, as influenced by the hydrogen-ion concentration, depend upon both the organism and the medium.

(5) It is not until a hydrogen-ion concentration of  $P_H$  1.5–2.5 is reached that inhibition of germination is evidenced. The limits on the acid side are comparatively narrow and constant. Those on the alkaline side are very diverse, varying with the organism and with the medium.

(6) Nutrition is an extremely important factor in the germination of fungous spores. In solutions of mannite, Czapek, and peptone, the relations of germination to hydrogen-ion concentration are more or less comparable for each particular organism; in beet decoction and "water HCl or KOH," on the other hand, results differing decidedly from those furnished by the previously mentioned solutions are obtained.

(7) Solutions of "water  $H_3PO_4$  and NaOH," followed by those of mannite, undoubtedly give the smallest range of germination and the lowest percentages of germination. Solutions of bacto-peptone give very similar results to those developed in solutions of mannite, except that germination is better and is extended slightly more to the alkaline side. Czapek's full nutrient solution generally exhibits ranges similar to those furnished by the peptone solutions, but the curves of germination differ. In the Czapek solution, two maxima usually occur: a primary one between  $P_H$  3.0 and 4.0 and a secondary one between  $P_H$  6.0 and 7.0. Of all the media employed, beet decoction



gives the best germination and the widest range of germination. Germination in this medium, as well as in "water HCl or KOH," occurs freely under conditions of active alkalinity, producing inhibition of germination in the other media, and germination is stimulated to such an extent that the maxima cover relatively wide zones. The same may be said of the peptone medium, though the magnitude and the frequency are less. The other media, namely, mannite, Czapek's solution, and water, exhibit distinct maxima in the form of sharp peaks.

(8) Mycelial growth and development in the most acid and alkaline cultures permitting germination are relatively more feeble and scant, as determined visually, than in cultures possessing greater departures from the inhibiting concentrations. Rudimentary germ tubes or knob-like projections frequently occur in the most alkaline cultures, and abnormally and irregularly shaped germ tubes generally develop in the most acid cultures.

(9) Comparing equal concentrations of H and OH ions, the OH ions appear to be relatively more toxic to the spores studied than H ions. The toxicity of H ions is fairly independent of the other conditions studied, while that of the OH ions tends to be more or less variable or antagonizable, according to the composition of the medium.

(10) A change in reaction of the medium may or may not occur during germination, and, where shifts in the hydrogen-ion concentration do occur, the magnitude and nature of these depend upon the fungus, the medium, and the initial reaction. While growth stages are marked by pronounced changes in reaction of the medium, *A. niger* produces no changes in reaction while germinating in the solution of mannite and in Czapek's medium, but does produce certain shifts towards neutrality in the alkaline cultures of the beet decoction. *B. cinerea* induces no changes in the reaction of the medium while germinating in solutions of mannite, Czapek's solution, "water  $H_3PO_4$  and NaOH," and "water HCl or KOH" but does produce a change in the beet decoction similar to but less than that of *A. niger*. The only conspicuous change brought about by germinating spores of *Fusarium* sp. is in "water HCl or KOH," all of the cultures giving favorable germination exhibiting a shift in reaction towards neutrality or slight alkalinity.

(11) The expanded range of germination under conditions of active alkalinity in such solutions as beet decoction and "water HCl or KOH" might appear on first glance to be correlated with buffer effects. Buffer action in these solutions is feebly present in the beet decoction and totally absent in the water. This is, no doubt, an important factor, but the experimental results here obtained seem hardly to justify an explanation entirely on such a basis. The fact that germinating spores of *B. cinerea* produce changes in reaction in the beet decoction medium and not in the "water HCl or KOH" is very striking.

(12) Various additions of sugar beet decoction to solutions of mannite pronouncedly stimulate germination with the two organisms under experimentation, namely, *A. niger* and *B. cinerea*. The percentages and the range of germination approach those offered by beet decoction alone, and no manifested resemblance to those furnished by the mannite alone is recognizable.

(13) It would seem, therefore, that germination in the beet decoction is stimulated by some special substance or peculiar set of conditions, either or both of which are totally or partially absent in the synthetic culture media.

(14) With increase in length of intervals of incubation, the relations of germination to hydrogen-ion concentration, other than a general increase in magnitude of all germination quantities and a frequent expansion of inhibitory limits, remain practically the same.

(15) The curves of germination for any organism are practically identical, whether incubated at a temperature representing a provisional optimum or at 4-5° C. above or below such an optimum. Germination often occurs feebly in cultures possessing a hydrogen-ion concentration closely approaching that of inhibition, and, if differences are manifested in the favorable ranges the tendency is for germination to occur over the widest range at the optimum temperature.

(16) The data here developed and presented, it is felt, are sufficient to change materially the previously prevailing view concerning the relation of germination of fungous spores to acid and alkaline media, as well as to be of fundamental importance in any future study of fungicides or spray mixtures.

The writer takes pleasure in acknowledging his indebtedness and extending his sincere thanks to Doctor B. M. Duggar for suggesting and directing the problem and for kindly criticism and advice throughout its prosecution; and to Doctor George T. Moore for the privileges and facilities of the Missouri Botanical Garden.

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## THE SIZES OF THE INFECTIVE PARTICLES IN THE MOSAIC DISEASE OF TOBACCO

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### INTRODUCTION

The present investigation is one of a series of studies in progress or proposed with the idea of gaining further information concerning the constitution and behavior of the causal agency in the mosaic disease of tobacco or other mosaic diseases. We have undertaken this work with the feeling that all facts tending to throw new light upon any physical or chemical characteristic of the agency concerned might be helpful in the study of some or all mosaic diseases, and likewise, perhaps, in the study of ultramicroscopic agencies causally related to certain human and other animal diseases. The term agency rather than organism is employed because it is hoped to avoid any possible prejudice to the direction in which such research may lead. It is distinctly felt that any assumption tacitly ascribing such diseases, because infectious, to organisms of the known or usual types may serve in the end to restrict rather than broaden the investigation. The term "virus" will be used in this paper interchangeably with agency.

It is, we believe, more frequently stated that the active agency

in the mosaic disease of tobacco is a filterable virus, the "contagium vivum fluidum" of Beijerinck ('98). Confirmation of Beijerinck's porous filter experiments is not lacking. On the other hand, the agency in this disease has been found to be held back, or non-filterable, when certain filters are employed. Experiments establishing the last-mentioned fact have been contributed by the work of Allard ('16) and also for the cucumber mosaic by Doolittle ('20). All too frequently, it would seem, our knowledge respecting the particles or individuals of the so-called filterable organisms has been chiefly the fact of the passage of infective particles through some bacteriological filter, more particularly the Chamberland or the Berkefeld, with no particular effort to effect a more precise standardization of both the filters permitting the passage of such particles and of those filters restraining them, so as to permit a more definite measurement of the particles concerned.

In this work some of the methods of ultrafiltration have been employed. In general, the method or technique of the experimentation may be divided into 3 phases: (1) filtration (or diffusion) of diseased juice through various ultrafilters, (2) inoculation of healthy plants with the filtrates obtained, and (3) the standardization of the filters by a determination of their capacity to permit or prevent the passage of colloidal particles of known, or approximately known, sizes.

### PRELIMINARY EXPERIMENTS

Preceding a discussion of the later work under 3 headings corresponding to the 3 phases, or aspects, above noted, it seems well to report certain preliminary data, secured during the previous year, which led to the more definite formulation of the chief experimental work reported in this paper. The preliminary experiments consisted of: (1) a filtration test of infected juice through a Livingston spherical atmometer cup, (2) filtration through layers of 1.5 and 3.0 per cent agar, (3) diffusion through Schleicher and Shüll parchment diffusion shells.

In these preliminary experiments with the atmometer the filter cup was partially filled with the juice, and suction was

then applied, a colorless filtrate being obtained with a pressure of about .5 atmosphere or more. The agar filtrates were obtained in the first instance by covering a Buchner funnel with filter-paper and then pouring on and congealing a layer of the agar to a depth of about 3 millimeters, being careful also to coat the sides of the funnel to a height that would be greater than the depth of the juice employed. Suction was then applied as before; filtration, however, was extremely slow. In the other case a cylindrical porous atmometer tube was partially filled with the melted agar, then by revolving the filter in a position almost horizontal and subsequently rapidly revolving it on a block of ice, as in the preparation of an Esmarch rolled plate, a layer of the agar was deposited throughout the length of the cylinder. In the case of the diffusion shells these were filled about half full with the diseased juice and then immersed to the depth of the inner liquid in small beakers of sterile distilled water. These were left for a period of 4 days at a temperature of about 18° C. in order that slow diffusion might proceed. The utmost care was used to prevent contamination of the exterior of any of the vessels employed. Inoculation experiments were made from each of the above tests, as indicated in the following outline.

TABLE I

INFECTION OF TOBACCO PLANTS WITH MOSAIC DISEASE AFTER  
FILTRATION OR DIFFUSION OF THE DISEASED JUICE

| Experiment number | Nature of filter or diffusion shell | Source of infection    | Number of plants diseased |
|-------------------|-------------------------------------|------------------------|---------------------------|
| 1                 | Spherical atmometer cup             | Filtrate               | 10                        |
| 2                 | Control                             | Control (dt. water)    | 0                         |
| 3                 | 1 5% agar layer                     | Filtrate               | 0                         |
| 4                 | 3 0% agar layer                     | Filtrate               | 0                         |
| 5                 | Parchment shell A                   | Liquid outside of tube | 0                         |
| 6                 | Parchment shell A                   | Liquid inside of tube  | 10                        |
| 7                 | Parchment shell B                   | Liquid outside of tube | 0                         |
| 8                 | Parchment shell B                   | Liquid inside of tube  | 9                         |

In the above experiments 10 tobacco plants were inoculated in each case. These were thrifty young plants of a common



variety, Kentucky burley. The inoculations were made on March 2 and final notes were taken March 16, though the plants were actually observed until April 11. No observations were made on temperature and humidity, but conditions in the greenhouse were such as to encourage rapid growth. These experiments were conclusive in showing that the virus or disease agency does not under our filtration conditions penetrate through agar of the consistencies employed, nor does it diffuse through a parchment membrane. On the other hand, the infected particles pass readily through the spherical atmometer cup. In this connection it should be observed that while the diffusion experiments lasted for a period of 4 days it has been shown (Allard, '16) that there is little, if any, lessening of pathogenicity in solutions subjected to more or less fermentation. The fact that the infected juice from within the diffusion shells invariably induced the disease is sufficient evidence that the growth of foreign organisms was not a factor worthy of consideration. The method of inoculation employed in the above experiments was the same as that described below for the more elaborate work here reported, and the reader is referred to the later description for the method employed.

It should be stated that several of the porous spherical atmometer cups have been tested in this laboratory under similar conditions and have been found invariably to prevent the passage of vegetative cells or of spores of *Bacillus subtilis*, and the subsequent results will show that this particular filter possesses finer pores than the Mandler diatomaceous filter. The indications furnished by Beijerinck as to the capacity of the virus to pass certain porous filters was again confirmed. On the other hand, Beijerinck claims a very slow diffusion, or penetration, of the virus into agar. The concentration of the agar is not noted. For the present the writers are unable to discuss the merits of this claim, since our own experiments represent direct filtration results, and the agar employed was probably denser than that used by Beijerinck.

#### FILTRATION OF THE DISEASED JUICE THROUGH ULTRAFILTERS

After the preliminary work reported above it was clear to the writers that it would be desirable to filter the diseased tobacco

juice through each of a series of porcelain or other filters of fairly well-determined porosity which might be subsequently standardized in a definite manner; but at the time no such series of filters could be found. Celloidin membranes did not seem to offer the range of porosity required. A little experimentation with rate of water flow, however, indicated that no inconsiderable range of possibilities was available in the form of the ordinary porcelain filters and atmometers of the laboratory. Accordingly, a series of filters was arranged consisting of a Mandler filter, a porous spherical atmometer cup, 2 cylindrical atmometer tubes, 2 cylindrical atmometer tubes infiltrated with precipitation films of  $\text{Al}(\text{OH})_3$ , and 2 specially prepared celloidin membranes. Considerable preliminary work led to the selection of this series. It may be well also to indicate that the particular spherical atmometer cup used in this work proved to be the only one possessing pores noticeably finer than the average of these cups. This cup was one of the earlier ones distributed for work in atmometers.

*Filters employed.*—The porcelain filters were, where necessary, thoroughly cleaned and all were boiled in distilled water prior to use. The Mandler filter employed was No. 5090 of the Arthur H. Thomas catalog,  $2\frac{1}{2}$ – $5\frac{1}{8}$  inches, tested to 6–12 pounds air pressure without passing air bubbles. The cylindrical filters impregnated with  $\text{Al}(\text{OH})_3$  were prepared in the following manner: The filter tubes were filled with 5 per cent  $\text{AlCl}_3$  and after allowing time for this to penetrate the walls thoroughly the tubes were suspended in beakers of 1 per cent  $\text{NH}_4\text{OH}$  until it appeared that the alkali had penetrated the cup, shown by a slight turbidity. The tubes were then carefully rinsed.

The celloidin membranes were prepared according to the method of Brown ('15) by which films of relatively great permeability can be obtained. The membranes were formed on the inside of beakers. An 8 per cent solution of Schering's celloidin in an equal volume of ether and absolute ethyl alcohol was poured into a beaker and allowed to drain over another beaker for 10 minutes. The beaker was then immediately immersed in distilled water. After about a minute the membrane was loosened from the sides of the beaker, washed in the water

for a short while, and allowed to dry over night at laboratory temperature. Since a very permeable membrane was desired, the film was put into 96 per cent ethyl alcohol for 24 hours at 20° C. and then thoroughly washed in water for a day. The films were cut into sizes large enough to fit over the broad end of a thistle tube. Tests of these membranes for leakage by the air bubble method were concurrent with the filtration experiments.

*Preparation of the juice from diseased leaves.*—A simple standard method, long in use in this laboratory for preparing the infected juice to be employed in experimental work, was adopted. This consists in pulping a known weight of the diseased leaves in a large mortar with a heavy pestle, then adding an equal weight of water and continuing the pulping until the leaf tissue is thoroughly crushed. The material is then filtered through cotton on a Buchner or ridged funnel. This diluted juice is used directly in the inoculation experiments.

*Filtration of the juices.*—In these experiments it was necessary to use every precaution possible to prevent accidental contamination of surfaces or vessels that might come in contact with the filtered juice. It was soon found that this could best be done by lowering the wet filter into the vessel containing the diseased juice to a suitable depth and then drawing the filtrate into the tube, rather than to draw the current from within outward. By the method indicated, as soon as sufficient filtrate had been drawn into the filter cup or tube, the filtration was stopped, and with sterile pipettes a quantity of the clear filtered juice was taken from within the cup and placed in clean vessels for use in inoculation.

With the various porcelain filters the water pump reduced the air pressure to 1/15–1/30 of an atmosphere. The filtrate was rapidly obtained in the case of the Mandler filter and also very nearly so rapidly in the case of the spherical atmometer cup. In fact, the time required to obtain a sufficient amount of the filtered juice was about 15 minutes with the spherical atmometer cup, and 30–45 minutes with the cylindrical ones. According to all the evidence at present available, such differences in pressure as were used do not materially influence the size of the

particles which may pass through, but primarily the rate of passage. The writers feel that it may be necessary to determine carefully the influence of the time interval; but since in these experiments comparative rather than fundamental results were desired, the phase of the filtration problem just referred to has not been experimentally studied.

With the celloidin membrane it was necessary to filter very cautiously so that a longer period of time at a pressure of 0.8 atmosphere was given. In this case, too, the membrane was fastened over the bell of a thistle tube. The diseased juice was then added through the tube, and the thistle tube—with the stem of the latter inserted through a rubber cork—was placed in a wide-mouthed bottle and lowered almost to the bottom, sufficient water being added to the bottle to just cover the membrane. Aspiration was then applied to the bottle through a second tube entering to just below the surface of the cork.

#### INOCULATION EXPERIMENTS WITH FILTERED JUICES

*Technique of inoculation.*—All inoculations were made by injuring the surface of the growing plant in 3 different areas, one from near the growing tip, one at the base of a young leaf, and another farther down the stem, or in the case of younger plants, just above the surface of the ground. These injuries were made with a needle or a fine pointed scalpel and in each case a drop of the infected juice was smeared over the injury and somewhat worked into it. This type of injury proved generally more effective than merely rubbing the stem or leaf as has been done in some cases. It was generally found advisable to make the inoculations in the late afternoon, the greenhouse being thoroughly watered afterward so as to prevent a too rapid drying of the injured surfaces.

Since there was some danger that the operator handling the filtration apparatus might come more or less in contact with particles of the diseased juice it was arranged that all inoculation work should be carried out by a different operator. Moreover, in most cases the different inoculation experiments were made by different operators. Where this was not possible every

precaution was taken with reference to contact with the clothing or hands. Between different inoculations the hands were washed with soap and water, then washed or rinsed with 1-500 formaldehyde, which has been found an effective antiseptic for the purpose, although when added to the juice in this concentration it is relatively ineffective.

*Results of inoculation experiments.*—There are given in table II the results of a series of inoculation experiments, with the filtered juices already described, conducted during November, 1921. In accordance with the indications previously given the inoculations were made on plants about 3 months old, which had been grown under greenhouse conditions and at this stage were in 5-inch pots. Good growing conditions were maintained throughout the experiment, since it has been repeatedly shown in our work that such conditions are favorable for most rapid production of unmistakable symptoms of the mosaic disease.

TABLE II

INOCULATION EXPERIMENTS MADE ON HEALTHY TOBACCO PLANTS,  
WITH FILTERED JUICES OBTAINED FROM PLANTS AFFECTED  
WITH THE CHARACTERISTIC MOSAIC DISEASE

| Exp. No. | Number of plants | Source of the inoculation                          | No. of plants with mosaic after 18 days |
|----------|------------------|--|---|
| 1        | 20               | Filtrate, Mandler filter                           | 19                                      |
| 2        | 20               | Filtrate, spherical atmometer cup                  | 18                                      |
| 3        | 20               | Filtrate, cylindrical atmometer tube A             | 1                                       |
| 4        | 20               | Filtrate, cylindrical atmometer tube B             | 0                                       |
| 5        | 20               | Filtrate, Atm. C. infilt. with Al(OH) <sub>3</sub> | 0                                       |
| 6        | 20               | Filtrate, Atm. D. infilt. with Al(OH) <sub>3</sub> | 1†                                      |
| 7        | 20               | Filtrate from celloidin membrane E                 | 1†                                      |
| 8        | —                | Filtrate from celloidin membrane F*                | —                                       |
| 9        | 20               | Control, juice from diseased plant                 | 19                                      |
| 10       | 20               | Control, distilled water                           | 0                                       |

\* This membrane leaked and no inoculations were made.

† These two plants exhibited pronounced symptoms of mosaic in so short a time after inoculation that they are thought to have been accidental contaminations.

From the results obtained it was clear that particular interest would attach to the spherical atmometer cup and to the filter

of next lower porosity, which proved to be the cylindrical atmometer A. After the standardization was carried out, as discussed later, bearing out the importance of the work with these 2 cups a second series of inoculations was made with new filtrates of diseased juice through these 2 cups. Twenty tobacco plants were inoculated with each filtrate and numerous uninoculated controls were kept in adjacent plats. Between 10 and 18 days after the inoculations 19 plants developed the disease among those inoculated with the spherical cup filtrate and 5 plants became diseased from the filtrate of the cylindrical cup A. Thus the previous test was admirably confirmed and even better indications were afforded that a small number of infected particles pass the cylindrical atmometer cup.

#### STANDARDIZATION OF THE FILTERS

In attempting to standardize the filters which had been employed in this work there was the possibility of using the same filter after a thorough cleansing, or the possibility of employing a similar filter assumed to be of equal porosity. It became evident that direct standardization of the original filter employed was essential where this could be done without fear of change or injury. Consequently the first step in the standardization involved a thorough cleansing of the filters employed. The standardization process was delayed until after the results of the inoculation in order to limit the amount of unnecessary work. From the inoculation experiments it was clear that the sizes of the infective particles must lie between the pore sizes of the spherical atmometer cup used and that of the most porous cylindrical tube A, and probably close to the pore sizes of the latter. At the time we had no idea of the relation of these sizes, and had not the subsequent standardization experiments indicated that these two pore sizes were sufficiently close together, it would have been necessary to seek further for a porous filter of intermediate pore dimensions.

To avoid difficulties arising from adsorption or from the possible action of electrolytes derived from the filters, it was determined to use organic sols rather than metallic sols for

standardization purposes. It was, however, with some regret that the use of gold sols was then considered undesirable, since the sizes of the particles in such solutions have been so well determined. In undertaking the standardization work it seemed best to use at the outset colloidal solutions that might represent extremes in sizes and then to narrow the field down to those that might correspond more nearly with the particles of the mosaic disease. Accordingly, a solution of dextrin was first used, since the particles represent extreme smallness in colloidal solutions, and moreover the filtrates could be readily tested by the simple iodine method. Filtration experiments with a 1 per cent dextrin solution indicated that these particles passed freely through all of the standard unimpregnated porcelain cups employed. A small quantity of dextrin passed the cylindrical cup C, impregnated with  $\text{Al}(\text{OH})_3$ , and none passed the other cup so impregnated.

In the next test milk free of fat was employed with a view to determining the size relation between the mosaic disease particles and casein in milk. The milk was first filtered through the spherical atmometer cup and it was found that this filter prevents entirely the passage of casein. The filtrate was a clear solution containing no demonstrable quantity of casein. It was now necessary to utilize a larger colloidal molecule for standardization than dextrin, and yet a molecule considerably smaller than casein in milk, thus hemoglobin was selected.

The hemoglobin employed was a preparation made by standard methods from ox blood. As soon as the ox blood was drawn neutral potassium oxalate to make 0.2 per cent was added in order to prevent clotting. A measured quantity of the blood was then distributed in centrifuge tubes and centrifuged, the supernatant serum being drawn off and the known volume of corpuscles thoroughly washed 4 times with a physiological salt solution (0.9 per cent  $\text{NaCl}$ ). An equal volume of distilled water was added to luke the corpuscles, after which the solution was again centrifuged to remove fibrin and stroma. The red supernatant colloidal solution was finally diluted so as to contain 1 per cent hemoglobin, estimating the original hemoglobin blood content at 12 per cent. For this work it was not necessary,

of course, to dialyze or otherwise further purify the product, as was requisite in the type of studies pursued by Bottazzi ('13), Reichert ('09), and others.

For ultrafiltration work hemoglobin has been recognized as a product of exceptional value. By any standard method of preparation it would seem that the particles are of fairly uniform size, so much so that it was employed by Bechhold in standardizing and designating the porosity of his gelatin filters. Nevertheless, the actual sizes of the particles do not seem to have been determined. In one of his papers Bechhold ('07) indicates that the particles must average a little less than 20  $\mu\mu$ , being fairly comparable with "Kollargol (koll. Silber v. Heyden)". In his text ('19), moreover, the same author places them at smaller than the particles of 1 per cent gelatin and larger than serum albumen, which would indicate a measurement somewhat greater than 30  $\mu\mu$ . Later in the same work (p. 111) he indicates the sizes of hemoglobin particles at 33–36  $\mu\mu$ . The diameter of the hemoglobin molecule has been given as 2.3–2.5  $\mu\mu$ .

The tests with the standardized hemoglobin solution yielded results both satisfactory and illuminating. Through the Mandler filter with the usual time interval mentioned the filtrate was a very deep red, yielding no appreciable dilution of the hemoglobin. Through the spherical atmometer cup the filtrate was still very red, indicating that relatively few particles of the hemoglobin were held back. Through the cylindrical atmometer cup A there was a very slight passage of hemoglobin particles, while through the cylindrical tube B and both tubes impregnated with  $\text{Al}(\text{OH})_3$  there was no passage of hemoglobin particles whatsoever in these tests.

Further it may be of interest to state that the spherical atmometer cup referred to above permitted approximately 50 per cent of the gelatin particles to pass through the filter from a 1 per cent solution of gelatin. The amount passing through was determined colorimetrically in comparison with the original solution by means of the Biuret test. The gelatin solution was prepared by adding gelatin to the boiling water and then immediately cooling to room temperature.



## DISCUSSION

From the results presented it would seem clear that with approximately equal pressures and equal time intervals the infective particles of the juice of tobacco plants affected with the mosaic disease possess about the same capacity to pass through the pores of porcelain filters as do the colloidal particles of fresh hemoglobin prepared by standard methods. No determinable dilution or loss of infectivity of the tobacco juice was occasioned by filtration through the spherical atmometer cup used in these experiments. On the other hand, a dilution of approximately 50 per cent resulted when a 1 per cent gelatin solution was filtered in the same cup. The sizes of the infective particles would therefore appear to be considerably less than those of gelatin particles, and since the particles of gelatin are not apparently very much larger than those of hemoglobin the conclusion is further strengthened that the infective particles here in question have about the size relations of fresh hemoglobin. In considering the estimated size of hemoglobin particles referred to previously in connection with the work of Bechhold it should be pointed out that Bechhold seems to have worked with dried preparations of hemoglobin, and it is perhaps to be expected that these would be larger rather than smaller than those of the fresh product. All indications are that, in general, a relatively freshly made colloidal solution possesses particles more uniform in size, and this idea is tentatively accepted. Assuming that at most the hemoglobin particles worked with may have possessed a diameter of  $30\mu$ , more or less, and that the average small diameter of bacterial plant pathogens is around  $1000\mu$  (some being as low as 500 and others as large as  $1500\mu$ ) we have 30:1000 to express roughly the diameter relations of mosaic disease particles in comparison with bacterial plant pathogens. On the basis of this average relation it is interesting to note that the volume relation would be about as follows: 1:37,000, or about 26:1,000,000, assuming that in each case we may treat the bodies as spherical structures.

The results of the filtration experiments have directed the attention of the writers to the possibility of the existence of

minute organisms or propagative parts of organisms in the soil or in other products which are commonly the seat of varied bacterial activities. While this has been previously pursued in certain directions an investigation of one important aspect of the problem has been undertaken. This work will be reported upon in a subsequent paper.

No reference has thus far been made to two recent reports by Kunkel which are of particular interest in this connection. In the earlier paper Kunkel ('21) has studied cytologically the tissues of corn affected with a mosaic disease and he reports, describes, and figures a foreign body believed to be a living organism invariably present in the diseased cells. The distribution of this body is found to correspond with the distribution of the light green color areas in the diseased leaves. While no proof has been afforded that these bodies are etiologically related to the corn mosaic, or even that they are living structures, it is suggested that they may be more or less analogous to the Negri bodies in the brain cells of animals suffering from rabies. In a more recent note Kunkel ('22) has associated ameboid bodies with the *Hippeastrum* mosaic, this host plant being a member of the *Amaryllidaceae*. Analogous bodies have not thus far been mentioned by those who have studied the mosaic disease of tobacco histologically or cytologically.

It is of course not certain that the mosaic diseases of these monocotyledonous plants are caused by organisms or agencies similar to those inducing the mosaic of tobacco. At the present time either possibility may be entertained. Even should an ameboidal structure be found in the cells affected with mosaic disease of tobacco and etiologically associated therewith, interest in the filtration experiments would remain. Whatever might be the size relations of such an organism in the uninjured cell, its behavior under filtration would indicate that relatively minute colloidal particles of the body are capable of reproducing it. A discussion of theoretical aspects is reserved until further experimental work has been done.<sup>1</sup>

<sup>1</sup>Since the above was written the attention of the writers has been drawn to an article previously overlooked on a filterable virus, as follows: Andriewski, P. L. 'ultra-filtration et les microbes invisibles. Centralbl. f. Bakt. I. 75: 90-93. 1914. Using

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the virus of the "peste des poules," this investigator compared the ultrafiltration of this disease agency with hemoglobin and serum albumin, all filtrations being made through a graded series of collodion membranes. It was determined that the particles of the virus were smaller than hemoglobin and about the size or somewhat smaller than serum albumin. In discussing actual sizes, however, he seems to confuse the sizes of colloidal particles of hemoglobin with the sizes of molecules. Nevertheless, his conclusion is to the effect that this virus cannot be formed of cells similar to those of plants and animals at present known.

## TILLETIA TEXANA IN MISSOURI

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While looking over smut collections in the herbarium of the Missouri Botanical Garden the writer came upon a collection on a wild grass, *Hordeum pusillum* Nutt., common in Missouri and in adjoining regions, which is of particular interest. This smut has apparently been reported only once before, from Texas, the type locality, and since the original description was based on but one collection a brief description of the Missouri material should be of some assistance in fixing the identity of this species as well as calling attention to a new host and a new locality.

This smut is of the covered type, the glumes remaining intact while the ovule is more or less completely replaced by the spore mass, and, like other smuts of the covered type, is apt to be overlooked. It was collected by C. H. Demetrio, near Emma, Saline County, Missouri, on June 20, 1896, and it is worth noting that while the host of the type collection is *Hordeum nodosum* L. (*H. pratense* Huds.) the collection under discussion is on *H. pusillum*.

Clinton's description of *Tilletia texana* Long (Jour. Myc. 8: 149. 1902), appearing also in the same author's monograph of the North American Ustilaginales, portrays well the collection at hand. The following additional notes may be of interest. The attacked ovules are considerably enlarged, often assuming two or three times the width of the normal kernels, and instead of appearing straw-colored they are of a grayish green external appearance. Internally they present an agglutinated light-reddish brown spore mass, as Clinton states. His description of the spores, including color, shape, markings, size, etc., might have been written for the Missouri material. I find the same characters that he describes. In addition it should be noted that the hyaline envelope is 2.5-3.5  $\mu$  thick and that many of

the spores show a small apiculus which at times is replaced by a slender thread-like, colorless hypha. This apiculus or slender thread simulates the aspects of a pedicel; at any rate, it is quite likely to be the point of attachment to the stromatic mass. Some of Lutman's figures (Trans. Wis. Acad. Sci. 16: 1191-1244. 1910), depicting the manner in which resting spores are developed in some smuts, would indicate that some such method of attachment of spore to the stroma is not uncommon. May this be regarded as a step culminating in the development of a true pedicel such as numerous rusts possess?

In Clinton's description it will be noted that he is not certain of the maturity of his material, and particularly in connection with the light orange-yellow color of the spores, he says: "Appearing as if somewhat immature." The Missouri material shows the same color and in the mind of the writer there is little doubt of the maturity of this material. In this region *Hordeum pusillum*, the host, is one of the grasses which appears early and usually matures during May or the first half of June. By the end of June this grass begins to disappear and is gradually supplanted by later developing grasses. As Demetrio's collection was made on June 20, there is little doubt that the host as well as the fungus must have reached maturity. The fact that other smuts, closely related to this species, are also light-colored should leave little doubt on this matter.

The relationship of *Tilletia texana* to other species is worthy of consideration. Besides this species four others of the genus *Tilletia* have been described on various species of *Hordeum*. They are *T. Hordei* Körn., *T. Trabuti* Jacz., *T. Panicicii* Bub. & Ranojevic, and *T. Bornmulleri* Magn. Clinton has already called attention to the difference between *T. texana* and *T. Hordei*, namely, the reticulate markings of spores of the latter species. The other species likewise are said to have reticulate spores besides other diagnostic characters which are not possessed by *T. texana*. Indeed, species on other host genera show greater similarity to this smut. Besides *T. buchloena*, mentioned by Clinton, *T. Wilcoxiana* Griffiths and perhaps *T. Rauwenhoffii* Fisch. de Waldh. are closely related. *Tilletia Wilcoxiana* on *Stipa Hassei* in particular deserves attention. Besides the

characteristic hyaline membrane the spores of this smut also are light-colored as well as possessing other features in common with *T. texana*. The differences as noted in material in the herbarium of the Missouri Botanical Garden (collected by H. E. Hasse at Los Angeles, Cal., April 5, 1895) are in the smaller markings and in the somewhat smaller-sized spores of *T. Wilcoxiana*. Whether these differences denote unlike species or merely influences of unlike hosts on the same species is a question. Without a larger number of collections and without cross-inoculation experiments it would perhaps be best to consider them as two distinct species.



# SOME NORTH AMERICAN TREMELLACEAE, DACRY- OMYCETACEAE, AND AURICULARIACEAE

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In 1899 I compared the authentic specimens of tremellaceous fungi in the Schweinitz herbarium in Philadelphia with collections which I had accumulated while living in Vermont, where many of the Schweinitzian species are frequent. From time to time I have studied the types of species described by Berkeley and Curtis and by Peck and made comparisons with them. My deep interest in Professor Coker's recent work 'The Lower Basidiomycetes of North Carolina'<sup>1</sup> and in Mr. Lloyd's studies and comments on various species leads me to present the following notes:

## TREMELLACEAE

*Peziza conrescens* Schw. and *Tremella reticulata* (Berk. & Curtis) Farl. are white species of *Tremella*, growing on the ground, of which the former is so soft that it may possibly be confused by collectors with the white plasmodium of a Myxomycete. This species has a long north and south range, for I have one specimen collected by Langlois in Louisiana, which Patouillard referred to *Tremella fuciformis*; the original collection was made by Schweinitz in North Carolina and again near Philadelphia, when its basidiomycetous nature was recognized and it was published as *Dacryomyces pellucidus* Schw. This species is the *Corticium tremellinum* Berk. & Ravenel, collected by Ravenel in Georgia and referred to by Farlow in *Rhodora* 10: 10. 1908. My collection was made by a mountain roadside between Lake Dunmore and Silver Lake, Vermont, where several fructifications were growing up from the ground incrusting herbs. In cases where several herbs were near enough together so that a fructification was using them all as supports,

<sup>1</sup> *Elisha Mitchell Scientif. Soc. Jour.* 35: 113-182. *pl.* 23, 35-66. 1920.

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the fructification sagged by its own weight into cup-shaped form in the space between the several supports. It was such a form which led Schweinitz to publish the original collection as a *Peziza*. When incrusting only two stems the fructification sagged between the supports in the form of a whitish pellucid membrane. By its dependence for support of its mass upon herbaceous stems and by absence of projecting self-supporting lobes, *Tremella concrescens* is distinguishable at sight from *T. reticulata*, which is also white and grows on the ground but stands up a self-supporting, coralloid mass with many short cylindric branches. *T. reticulata* has been frequently collected in the northern United States from Vermont westward to Minnesota but with southern limit the North Carolina station given by Coker.

*Tremella fuciformis* Berk. is the third species of the group. This is a tropical species ranging from Brazil through the West Indies into the southern United States as far north as North Carolina. It has been collected but few times and has always been found growing on wood. There has been a tendency to confuse both *T. reticulata* and *Dacryomyces pellucidus*, the synonym of *T. concrescens*, with *T. fuciformis* but the growth from the ground, not wood, seems a reliable means of distinction, although there are additional features of distinguishing *T. fuciformis* when it has to be determined in the herbarium from dried specimens not accompanied by notes as to substratum. *T. fuciformis* dries with the upper portions white and the basal portion in the region in and near the wood ochraceous tawny; it agrees with *T. reticulata* in being self-supporting and branched, but in dried condition main trunk, main branches, and final branches are not at all cylindric but flattened into leaf-like form with branches at the margins of the main trunk and main branches and all in a common plane although more or less crisped by the great number of ultimate branches. There are differences between these three species in microscopic characters which are given in the following more complete descriptions with synonymy, etc.

*Tremella concrescens* (Schw.) Burt, n. comb. Plate 3, fig. 1.

*Peziza concrescens* Schweinitz, Naturforsch. Ges. Leipzig

Schrift. 1: 118. 1822; Am. Phil. Soc. Trans. N. S. 4: 171. 1832; Fries, Syst. Myc. 2: 53. 1823; Sacc. Syll. Fung. 8: 76. 1889.—*Dacryomyces pellucidus* Schweinitz, Am. Phil. Soc. Trans. N. S. 4: 186. 1832; Sacc. Syll. Fung. 6: 804. 1888; Morgan, Cincinnati Soc. Nat. Hist. Jour. 11: 94. 1888; Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 173. 1920.—*Corticium tremellinum* Berkeley & Ravenel, Grevillea 1: 180. 1873; Sacc. Syll. Fung. 6: 632. 1888; Massee, Linn. Soc. Bot. Jour. 27: 146. 1890; Farlow, Rhodora 10: 10. 1908.—An *Tremella vesicaria* of Lloyd, Myc. Writ. 5. Myc. Notes 60: 871. text f. 1486. 1919? Not *Tremella vesicaria* Bulliard.

Type: in Herb. Schweinitz.

Fructifications gelatinous, very soft, growing up from the ground and ascending, incrusting and supported by herbaceous stems between which the masses are suspended in various forms determined by distribution of the supports, often a whitish, semi-pellucid membrane, drying hard, horn-like, somewhat wood-brown, and more or less veined; basidia longitudinally, cruciately septate, subglobose,  $12-15 \times 10-12 \mu$ ; spores hyaline, even,  $8-9 \times 4\frac{1}{2}-6 \mu$ .

Fructifications 2-6 cm. high and broad.

On the ground by roadsides in woods, growing up and crescent with stems of plants and other parts. Vermont to Louisiana and in Missouri. July and August. Rare.

This species is characterized by its occurrence on the ground from which it rises by support of small stems and other objects, absence of branches of characteristic form, rather large, subglobose basidia, and the small spores. Lloyd's figure which I have cited does not show the usual aspect of fructifications of this species. The form C noted by Gilbert, Wis. Acad. Trans. 16: 1153. pl. 83. f. 22. 1910, seems to be *T. conrescens*.

Specimens examined:

Vermont: near Lake Dunmore, E. A. Burt.

Pennsylvania: near Philadelphia, Schweinitz, type of *Dacryomyces pellucidus* (in Herb. Schweinitz).

North Carolina: Schweinitz, type (in Herb. Schweinitz).

Georgia: Cotoosa Springs, Ravenel, 1754, type of *Corticium tremellinum* (in Curtis Herb.).

Alabama: *Peters*, 897 (in Curtis Herb.).

Louisiana: *A. B. Langlois*, 2973; St. Martinville, *A. B. Langlois*, 2087, under the herbarium name *Sebacina tremellosa* E. & E.

Missouri: St. Louis, *N. M. Glatfelter*, 229 (in Mo. Bot. Gard. Herb., 57674).

*T. reticulata* (Berk.) Farlow, *Rhodora* 10: 9. Ja. 1908; Gilbert, Wis. Acad. Trans. 16: 1152. *pl.* 83. *f.* 17-21. 1910; Sacc. Syll. Fung. 21: 455. 1912; Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 139. *pl.* 37; *pl.* 56. *f.* 12. 1920.

*Corticium tremellinum* var. *reticulatum* Berk. *Grevillea* 1: 180. 1873; Sacc. Syll. Fung. 6: 632. 1888; Masee, Linn. Soc. Bot. Jour. 27: 146. 1890.—*C. reticulatum* Berk. & Curtis in Cooke, *Grevillea* 20: 13. 1891.—*Tremella Clavarioides* Lloyd, Myc. Writ. 3. Myc. Notes, Old Species 1: 10. *text f.* 224. Ju. 1908.—*T. Sparassoidea* Lloyd, Myc. Writ. 6. Myc. Notes 61: 894. *pl.* 135. *f.* 1562. 1920, and Myc. Notes 62: *pl.* 145. *f.* 1646. 1920; Overholts, *Mycologia* 12: 141. *pl.* 10. *f.* 3. 1920.—*T. fuciformis* Atkinson, *Mushrooms*, 206. *text f.* 207, but not *T. fuciformis* Berk.

Illustrations: Atkinson, Coker, Gilbert, Lloyd, and Overholts, *loc. cit.*

Type: in Curtis Herb.

Fructifications gelatinous, rather firm, elastic, white, growing up from the ground in erect, branched, self-supporting tufts which are more or less fused together and anastomosing, with all parts usually hollow, finally becoming somewhat cinnamon-brown in the herbarium; branches somewhat cylindric, short, projecting, obtuse; basidia  $12 \times 8-9 \mu$ ; spores hyaline, even,  $6-10 \times 4\frac{1}{2}-6 \mu$ , as found in preparations of the hymenium.

Fructifications  $2\frac{1}{2}-8$  cm. high,  $3\frac{1}{2}-10$  cm. in diameter.

Growing on the ground in woods, Vermont to North Carolina and westward to Wisconsin. July to October.

*Tremella reticulata* is distinguished by its rising from the ground as a white, self-supporting, coralloid mass so firm and elastic that it can be bent, twisted, or compressed and the parts spring back into their original position.

Specimens examined:

Vermont: Grand View Mt., *E. A. Burt*; Middlebury, *E. A. Burt*.  
Pennsylvania: comm. by C. H. Peck under the name *T. vesicaria*; *Michener*, 1212, type of *Corticium tremellinum* var. *reticulatum*, (in Curtis Herb., 3942).

Minnesota: Minneapolis, *M. L. Whetstone*, comm. by F. Weiss, type of *T. Sparassoidea* (in Mo. Bot. Gard. Herb., 56256).

*T. fuciformis* Berkeley, Hooker's Jour. Bot. 8: 277. 1856; Linn. Soc. Bot. Jour. 10: 340. 1868; Sacc. Syll. Fung. 6: 782. 1888; A. Möller, Bot. Mitt. a. d. Tropen 8: 115. pl. 1. f. 5; pl. 4. f. 13. 1895; Farlow, Rhodora 10: 11. 1908; Lloyd, Myc. Writ. 5. Myc. Notes 55: 790. text f. 1188. 1918; Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 140. pl. 38, 56. f. 7. 1920.

Illustrations: as given above and Engl. & Prantl, Nat. Pflanzenfam. (I:1\*\*): 93. text f. 60 H.

Type: probably in Kew Herb.

Fructifications solitary or cespitose, gelatinous, rather tough, erect, white, repeatedly lobed or forked, with the peripheral lobes thin, flat, much crinkled or fluted, drying with the upper portion white and basal portion ochraceous tawny; basidia subglobose,  $7-10 \times 6-7\frac{1}{2} \mu$ ; spores hyaline, even,  $5-6 \times 4-4\frac{1}{2} \mu$ , as found in a preparation of the hymenium.

Mass of fructifications about 2 cm. high and 3-5 cm. in diameter in northern specimens, attaining larger size in Brazil.

On dead wood. North Carolina, West Indies, and Brazil. October to January.

*Tremella fuciformis* occurs on wood in a rosette-like mass of thin, crinkled, and fluted lobes, white and drying white except in the region of attachment to the wood where the color is ochraceous tawny; the basidia and spores are subglobose and small.

Specimens examined:

Cuba: *C. Wright*, 233 (in Curtis Herb.).

Porto Rico: Bayamon, *J. A. Stevenson*, 6765 (in Mo. Bot. Gard. Herb., 55655).

Other white species of tremellaceous fungi occurring on a wood substratum are *Exidia alba* and *E. candida*. *E. alba* is frequent in the middle west from Wisconsin southward to Ala-

bama along the northern range of *Tremella fuciformis*. *E. alba* was formerly confused with *E. albida* of Europe until Lloyd pointed out that the former is clearly distinct from any known white tremelline species of Europe by the presence of gloeocystidia in its hymenium. Lloyd included *E. alba* in the little-known Australian genus *Seismosarca* but I am reluctant to follow him in this respect, for since genera are merely rather natural groups of species of convenient size for taxonomic work, it seems unnecessary and a great pity to segregate already small genera on the basis of every positive character which would make a species noteworthy. *E. candida* is known so far from the state of Washington only.

The details of the above species are as follows:

***Exidia alba* (Lloyd) Burt, n. comb.**

*Exidiopsis alba* Lloyd, Myc. Writ. 4. Letter 44:8. 1913.—*Seismosarca alba* Lloyd, Myc. Writ. 5. Myc. Notes 45: 629. 1917; Myc. Writ. 6. Myc. Notes 65: 1045. *f.* 1928, 1929. 1921.

Fructifications large, cerebriform, subfoliaceous or with rounded convolutions, white or somewhat creamy, marginal portions discoloring in the herbarium to tawny olive and Sayal-brown and the more central regions approaching fuscous; gloeocystidia somewhat colored, cylindric, flexuous, up to  $30 \times 6 \mu$ ; basidia subglobose,  $10 \times 9 \mu$ ; spores hyaline, curved, even,  $9-10 \times 4\frac{1}{2} \mu$ ; edible.

Fructifications 1-4 cm. high, 2-10 cm. in diameter.

On dead wood. According to literature probably ranging from New York to Minnesota and southward to Alabama but known to me by specimens from Wisconsin to Alabama only. June to October. Frequent.

Within the basin of the Mississippi *E. alba* is the common species occurring in large, white or slightly creamy masses on dead wood; reference of collections to this species may be confirmed by presence of the conspicuous gloeocystidia when a bit of the hymenium is crushed in water under a cover glass. Dr. Glatfelter found this species so abundant in Forest Park, St. Louis, that he tested its edible properties, and he noted on the collection which was preserved that this species is "edible but not delicious."

## Specimens examined:

Wisconsin: Blue Mounds, *E. T. & S. A. Harper*, 868.

Missouri: Creve Coeur, *L. O. Overholts* (in Mo. Bot. Gard. Herb., 57678); St. Louis, *N. M. Glatfelter*, 49 (in Mo. Bot. Gard. Herb., 57677).

Alabama: Montgomery, *R. P. Burke*, 78 (in Mo. Bot. Gard. Herb., 13540).

*E. candida* Lloyd, Myc. Writ. 5. Myc. Notes 44: 620. text f. 880, 881. 1917.

Fructifications effused, somewhat pulvinate, with the surface tuberculate and having irregular folds, white or grayish, discoloring to bister in the herbarium when dry, and cracking and curling up from the substratum; basidia  $12-15 \times 10 \mu$ ; spores hyaline, even,  $12-13 \times 4-4\frac{1}{2} \mu$ , stated by Lloyd to be  $16 \times 8 \mu$ ; no gloeocystidia.

Fructification 2-5 mm. thick, spread out over areas 10 cm. and more in diameter.

On rotten *Corylus*. Washington. August.

This species is noteworthy by its broadly effused and relatively thin fructifications and spores at least twice as long as broad.

## Specimens examined:

Washington: Bingen, *W. N. Suksdorf*, 751.

In December, 1899, I studied the specimen of *Tremella aurantia* Schw. in Herb. Schweinitz in Philadelphia, before it had been examined by either Lloyd or Coker. I noted that it was on an oak limb which was also bearing *Stereum rameale*. The preparation which I have of a bit of the hymenium of this authentic specimen still shows the longitudinally cruciately septate basidia and subglobose, hyaline, even spores about  $10 \times 8 \mu$ . These dimensions do not exclude *Tremella mesenterica*, but the form and general aspect of the fructification and its less brittle structure made me regard *T. aurantia* as a species distinct from the latter. In the following March I received from Professor P. H. Rolfs, then of Clemson College, South Carolina, a fine specimen from that region which measured  $4\frac{1}{2} \times 3 \times 2\frac{1}{2}$  cm. high when fresh. This specimen agreed in all respects with my notes, preparations, and remembrance of the

authentic *T. aurantia* in Herb. Schweinitz and with the original description of this species which was based on specimens collected at Salem, North Carolina. Later in the year Professor Rolfs sent me another gathering of *T. aurantia*. These specimens from Professor Rolfs upon being split open proved to be white and fibrous-fleshy within, being cogenetic in this respect with *Naematelia encephala*, and they show this structure well at the present time, hence *Tremella aurantia* should be transferred to *Naematelia*. Coker has made this disposition of the species but under the name *Naematelia quercina* Coker. The descriptions and synonymy of this species and of the related *N. encephala* follow:

***Naematelia aurantia* (Schw.) Burt, n. comb.**

*Tremella aurantia* Schweinitz, Naturforsch. Ges. Leipzig Schrift. 1: 114. 1822; Am. Phil. Soc. Trans. N. S. 4: 185. 1832; Fries, Syst. Myc. 2: 213. 1823; Epicr. 588. 1838; Sacc. Syll. Fung. 6: 781. 1888; Lloyd, Myc. Writ. 3. Myc. Notes, Old Species 1: 11, with text f 225 doubtful. 1903.—*Naematelia quercina* Coker, Elisha Mitchell Scientific Soc. Jour. 6. 135. pl. 23, f. 1; pl. 58, f. 1-2. 1920; Lloyd, Myc. Writ. 35: Myc. Notes 64: 1024. 1921.—An *Sparassis tremelloides* Berkeley, Grevillea 2: 6. 1873? See Lloyd, Myc. Writ. 6. Myc. Notes 64: 1025. 1921.—Not *Tremella aurantia* of Farlow, Appalachia 3: 248. 1883, nor of Coker, Elisha Mitchell Scientific Soc. Jour. 35: 163. 1920.

Illustrations: Coker, *loc. cit.*

Fructifications a hemispherical or more elongated, cockscomb-shaped mass divided nearly to the substratum into a few—about 3-6—somewhat flattened and crumpled lobes, xanthine-orange (aurantiacus of Saccardo's 'Chromotaxia'), drying ochraceous orange to walnut-brown in the herbarium, and solid, fibrous, and whitish within when dried; basidia subglobose, longitudinally cruciately septate,  $15-18 \times 12-15 \mu$ , often about  $15 \mu$  in diameter; spores hyaline, even, subglobose,  $9-12 \times 8-9 \mu$ .

Fructifications when fresh up to  $2\frac{1}{2}$  cm. high by  $4\frac{1}{2} \times 3$  cm., contracting when drying to masses 5-7 mm. high by  $12-16 \times 6-12$  mm.

On dead wood of frondose species. New Jersey to South Carolina. December to March. Rare.

\*Coker has published that the color is orange-yellow inside and out except for a thin white membrane about 0.7 mm. from the surface which follows all the convolutions and gives a marbled appearance to the cut surface. In the three gatherings before me which have been kept in the herbarium 20 to 25 years, the whole interior is as whitish within in its dried condition as it is in *Naematelia encephala* from which *N. aurantia* is distinguished in aspect by its larger, orange-colored fructifications which are divided nearly to the substratum into a few large lobes and by its occurrence on dead wood and dead saplings of oak and other frondose species.

Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fungi, 1719, under the name *Naematelia encephala*—three of the fructifications comprising the specimen in Mo. Bot. Gard. Herb. copy are *N. aurantia* and the fourth is *Tremella mesenterica*; Ell. & Ev., Fungi Col. 1118, under the name *N. encephala*.

New Jersey: Newfield, J. B. Ellis, in Ell. & Ev., N. Am. Fungi, 1719, and Fungi Col., 1118.

North Carolina: Schweinitz, type (in Herb. Schweinitz).

South Carolina: Clemson College, P. H. Rolfs, 3, 1888.

*N. encephala* (Willd.) Fries, Obs. Myc. 2: 370. 1818; Syst. Myc. 2: 227. 1823; Epicr. 591. 1838; Hym. Eur. 696. 1874; Berkeley, Outl. Brit. Fung. 290. 1860; Sacc. Syll. Fung. 6: 793. 1888.

*Tremella encephaliformis* Willdenow, Bot. Mag. 2: 17. pl. 4. f. 14. 1788.—*Naematelia encephaliformis* (Willd.) Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 137. 1920.—*Tremella encephala* (Willd.) Persoon, Syn. Fung. 623. 1801; Myc. Eur. 1: 98. 1822; Engl. & Prantl, Nat. Pflanzenfam. (I: 1\*\*): 94. 1897.

Illustrations: Willdenow, loc. cit.; Stevenson, Brit. Hym.—2: 316. text f. 99. 1886; Smith, Brit. Basidiomycetes, 452. text f. 117. 1908; Brefeld, Untersuch. Myk. 7: pl. 8. f. 20. 1888.



Fructifications solitary or clustered, nearly sessile, pulvinate, plicate-rugose, solid, drying cinnamon to Natal-brown externally and white and fibrous within; basidia 12–15  $\mu$  in diameter; spores hyaline, even, subglobose, 8–10  $\times$  7–9  $\mu$ .

Dried fructifications 3–10 mm. in diameter, 3–5 mm. high.

On dead, fallen branches of coniferous species. Ontario to North Carolina. August. Rare.

*Naematelia encephala* has small fructifications which are nearly subglobose, scarcely more than rugose on the surface and not deeply divided; attachment to the substratum is usually by a point rather than by a broad resupinate surface; the substratum is pine or spruce in all specimens known to me.

Specimens examined:

Exsiccati: Berkeley, Brit. Fungi, 291; Krieger, Fungi Sax., 1008; Sydow, Myc. Germ., 58.

England: Berkeley, Brit. Fungi, 291.

Germany: Saxony, *H. & P. Sydow*, in Sydow, Myc. Germ., 58; Winterberge, *G. Wagner*, in Krieger, Fungi Sax., 1008.

Canada: Ontario, Temagami, *H. von Schrenk* (in Mo. Bot. Gard. Herb., 57052).

New Hampshire: Tuckerman's Ravine, *W. G. Farlow* (in Mo. Bot. Gard. Herb., 5352).

Vermont: Middlebury, *E. A. Burt*.

Under the name *Tremella nucleata* Schweinitz described a species of quite different structure from *Naematelia encephala* and *N. aurantia*. Fries transferred this species to *Naematelia* because dried specimens contain scattered, white, spherical or lens-shaped calcareous masses imbedded in the fructification. These masses were termed nuclei by Fries but they are not of organic nature, being merely concretions<sup>1</sup> of calcium oxalate present in the gelatinous fructification and quite different from the white fibrous structure which forms the interior of *N. aurantia* and *N. encephala*. Some species of *Eridia* contain calcareous masses similar to those of *T. nucleata*, and since the spores of the latter are of the elongated form by which in herbarium work we distinguish *Eridia* from *Tremella*, I transfer this species to *Eridia*, as follows:

<sup>1</sup> Topin, Rev. Myc. 25: 134. pl. 233. f. 21. 1903.

***Exidia nucleata*** (Schw.) Burt, n. comb.

*Tremella nucleata* Schweinitz, Naturforsch. Ges. Leipzig Schrift. 1: 115. 1822.—*Naematelia nucleata* (Schw.) Fries, Epicr. 592. 1838; Hym. Eur. 696. 1874; Berkeley, Outl. Brit. Fung. 290. 1860; Peck, N. Y. State Mus. Rept. 24: 83. 1872; Berkeley & Curtis, Grevillea 2: 20. 1873; Morgan, Cincinnati Soc. Nat. Hist. Jour. 11: 93. 1888; Sacc. Syll. Fung. 6: 793. 1888; Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 136. *pl. 23. f. 3; pl. 41. f. 1; pl. 56. f. 3-5.* 1920.—An *Exidia gemmata* (Lév.)?

Illustrations: Coker, *loc. cit.*

Type: in Herb. Schweinitz.

Fructification effused, plane, somewhat gyrose and undulate, white at first, shrinking to a membrane in drying and becoming tawny olive to mummy-brown and containing a few scattered, conspicuous, white, subglobose concretions of calcium oxalate about 1/5–1/3 mm. in diameter; basidia 8–12 × 6–8  $\mu$ ; spores hyaline, even, curved, 8–9 × 3–4  $\mu$ .

Covering areas 5 mm.–3 cm. in diameter, not thicker when dry than the imbedded concretions.

On fallen limbs of frondose species. Maine to Louisiana and westward to California; occurs also in Europe. September to March. Widely distributed but not common.

*Exidia nucleata* is noteworthy by fructifications so thin that they suggest a *Sebacina* but are gelatinous throughout and often elevated or pinched up in the center, by the tawny olive color assumed in drying, and by the more or less numerous, white, chalky, seed-shaped concretions. I know *Exidia gemmata* of Europe only by the specimen received under this name from Bourdot; this specimen agrees in all respects with our *E. nucleata*.

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 520; Ravenel, Fungi Car. 4: 82.

France: Allier, St. Priest, *H. Bourdot*, 12147.

Maine: Orono, *F. L. Harvey* (in Mo. Bot. Gard. Herb., 1733, 5353).

Vermont: Middlebury, *E. A. Burr*.

New Jersey: *J. B. Ellis*, in Ellis, N. Am. Fungi, 520.

North Carolina: *Schweinitz*, type (in Curtis Herb.).

Alabama: *Peters*, in Ravenel, *Fungi Car.* 4: 82.

Louisiana: St. Martinville, *A. B. Langlois*, *by*.

Michigan: Ann Arbor, *C. H. Kauffman* (in *Mo. Bot. Gard. Herb.*, 58674).

California: Santa Catalina Island, *L. W. Nuttall*, 524, 1012, comm. by Field Mus. Nat. Hist. Herb. (in *Mo. Bot. Gard. Herb.*, 57624, 57683).

Another Schweinitzian species is also noteworthy by containing more or less numerous, small, white, chalky concretions although not so noted by Schweinitz. This is his *Eridia spiculata*, a species of which I made gatherings in Vermont on rotting willow and other frondose species, growing from cracks in the bark.

*E. spiculata* Schweinitz, *Am. Phil. Soc. Trans. N. S.* 4: 185. 1832; *Sacc. Syll. Fung.* 6: 776. 1888; Coker, *Elisha Mitchell Scientif. Soc. Jour.* 35: 151. 1920.

Type: in Herb. Schweinitz.

Growing out from cracks in the bark in elongated masses, with crumpled, rugose surface, about 3 mm. high, 2-4 mm. wide, 10-12 mm. long, between sepia and clove-brown when wet, shrinking when dried to a thin, fuscous-black membrane with veined and wrinkled surface and now showing white, seed-like concretions  $\frac{1}{5}$ - $\frac{1}{2}$  mm. in diameter; basidia  $9 \times 6 \mu$ ; spores simple, hyaline, curved,  $9-10 \times 4 \mu$ .

On bark of fallen, decaying limbs of *Salix*, *Betula*, etc. Vermont to Pennsylvania. September to March.

*E. spiculata* and *E. nucleata* differ from other species of *Eridia* by containing small, whitish, seed-like concretions. *E. spiculata* is darker-colored than *E. nucleata*, much thicker, and with a crumpled surface. The surface was described by Schweinitz as papillate; perhaps he used the term in a broad way, for I fail to find true papillae either on the surface of the specimen in Herb. Schweinitz or of my collections.

Specimens examined:

Vermont: Lake Dunmore, *E. A. Burt*, two collections; Middlebury, *E. A. Burt*, two collections.

New York: Altamont, *E. A. Burt*.

Pennsylvania: Bethlehem, *Schweinitz*, type (in Herb. Schweinitz).

*Tremella colorata* Peck, N. Y. State Mus. Rept. 25: 83. 1873; Sacc. Syll. Fung. 6: 788. 1888.

I have not collected this species but the color reactions of the type are so remarkable that, if constant, they should distinguish the species from all others known to me. In the first place the ash bark and wood for a distance about the fructification are now, fifty years since the collection was made, still conspicuously stained vinaceous-drab as noted by Peck. Furthermore, in my microscopical, glycerin mount of this fungus, stained with Gruebler's alcoholic eosin and the color set with a trace of acetic acid, the basidia and hyphae are vinaceous-lilac instead of the brighter red usually given by the eosin. The basidia are spherical, 13–15  $\mu$  in diameter, longitudinally cruciately septate, mostly still immature although occasionally one may be found bearing slender sterigmata up to 30  $\mu$  long; only four spore-like bodies have been found; all are hyaline, simple, even, curved, two are  $7 \times 4\frac{1}{2}$   $\mu$  and the other two  $15 \times 6$   $\mu$ . It seems improbable that the spores are colored, globose, 12–15  $\mu$  in diameter, as published by Peck. Should an *Exidia* be collected having color characters and basidia as noted, comparison with the type as to other characters will probably demonstrate that it is *T. colorata* Pk.

*Tremella subcarnosa* Peck, N. Y. State Mus. Rept. 32: 36. 1879; N. Y. State Mus. Bul. 1<sup>2</sup>: 15. 1887; Sacc. Syll. Fung. 9: 258. 1891.

Examination of the type in N. Y. State Mus. Herb. shows that this fungus is not a Basidiomycete but rather one of the *Tuberculariaceae*.

So many species of *Tremellaceae* had been published as species of *Thelephora*, *Stereum*, and *Corticium* and were distributed under these genera in herbaria that I have already published<sup>1</sup> for the convenience of students of the *Thelephoraceae* an account of the central-stemmed tremelloid genus *Tremello-dendron*, the reflexed *Eichleriella*, and the resupinate *Sebacina*.

<sup>1</sup> Mo. Bot. Gard. Ann. 2: 731–770. 1915.

We have a few species of tremellaceous fungi which are hydroid in general aspect and belong in *Heterochaete*, a genus defined as follows:

**HETEROCHAETE** Patouillard, a genus of resupinate tremellaceous fungi whose species have the general aspect of species of *Odontia* with cystidia clustered in the granules and with the basidia longitudinally cruciately septate. Our North American species are *H. andina*, *H. gelatinosa*, *H. sublivida*, *H. microspora*, and *H. Shearii*—none of which are known from north of District of Columbia.

**Heterochaete andina** Patouillard & Lagerheim, Soc. Myc. Fr. Bul. 8: 120. pl. 11. f. 2. 1892; Sacc. Syll. Fung. 11: 144. 1895.

Illustrations: Patouillard, *loc. cit.*

Fructifications resupinate, effused, thin, adnate, drying cartridge-buff, with margin whitish, the surface bearing numerous small, sharp-pointed granules; in structure 60–75  $\mu$  thick, composed mostly of densely interwoven, hyaline hyphae of uneven outline,  $2\frac{1}{2}$ –3  $\mu$  in diameter, sometimes with hyphae slightly colored next to substratum; granules cylindric, 1201–50  $\mu$  high, 40–60  $\mu$  in diameter, containing an axile cluster of slightly colored or sometimes hyaline, granule-incrusted hyphae 3  $\mu$  in diameter which spread apart at the apex; basidia longitudinally septate,  $12-16 \times 6-9 \mu$ ; spores hyaline, even, curved,  $12-14 \times 4-7 \mu$ .

On dead fallen branches of frondose species. Florida, Louisiana, and West Indies to Ecuador. November to April.

*Heterochaete andina* has the aspect of a nearly white *Odontia* or resupinate *Hydnum*, from both of which it is distinguished by the longitudinally cruciately septate basidia. One of the Louisiana specimens cited below is from a gathering which was determined by Patouillard for Langlois as *H. andina*.

Specimens examined:

Florida: Cocoanut Grove, *R. Thaxter*, 93 (in Mo. Bot. Gard. Herb., 43920, and in Farlow Herb.).

Louisiana: Baton Rouge, *Humphrey & Edgerton*, comm. by C. J. Humphrey, 5710 (in Mo. Bot. Gard. Herb., 9982); St. Martinville, *A. B. Langlois*, 2855, 2988, and *ah*.

Porto Rico: Bayamon, *J. A. Stevenson*, 6303 (in Mo. Bot. Gard. Herb., 55085).

Mexico: Orizaba, *W. A. & E. L. Murrill*, 798 and 749 b (in Mo. Bot. Gard. Herb., 54614 and 54653, and in N. Y. Bot. Gard. Herb.).

*H. sublivida* Patouillard, Soc. Myc. Fr. Bul. 24: 2. 1908; Sacc. Syll. Fung. 21: 449. 1912.

*H. Burtii* Bresadola, Ann. Myc. 18: 51. 1920.

Fructifications resupinate, adnate, broadly effused, thin, drab-gray to light drab, the margin of the same color or paler; hymenium bearing more or less numerous granules or papillae with whitish tips; in structure 100–200  $\mu$  thick, composed of interwoven hyaline hyphae 2–2½  $\mu$  in diameter, and some masses of crystalline matter; granules 200–300  $\mu$  high by 100  $\mu$  in diameter at the base, composed of a few hyphae and much crystalline matter in masses; basidia longitudinally septate, 16–20  $\times$  8–10  $\mu$ ; spores white in collection on slide, flattened on one side, 8–10  $\times$  5–6  $\mu$ .

Covering areas 6 cm. and more long, 3 cm. and more wide.

On bark of decaying frondose wood. Louisiana and the West Indies. October to March.

This species has been confused in American mycology with *Grandinia ocellata*, from which it is distinct by its longitudinally septate basidia; it may be easily separated from our other species of *Heterochaete* by its livid (drab of Ridgway) color. Nearly twenty years ago I shared with Bresadola a specimen of this fungus received from Langlois. The interruption to correspondence by the war prevented my calling Bresadola's attention to the fact that a portion of another gathering, communicated by Langlois to Patouillard, was published by the latter as a new species, hence the synonymy.

Specimens examined:

Louisiana: St. Martinville, *A. B. Langlois*, 2882, cotype of *H. sublivida*, ~~the~~ cotype of *H. Burtii*, and at

Cuba: El Yunque Mt., Baracoa, *L. M. Underwood & F. S. Earle*, 371, N. Y. Bot. Gard., Fungi of Cuba.

Porto Rico: Campo Alegre, *J. A. Stevenson*, 6370 (in Mo. Bot. Gard. Herb., 55658).

*H. gelatinosa* (Berk. & Curtis) Patouillard, Soc. Myc. Fr. Bul. 8: 120. 1892; Sacc. Syll. Fung. 11: 144. 1895; Lloyd, Myc. Writ. 5. Myc. Notes 59: 857. *text f. 1439*. 1919.

*Kneiffia gelatinosa* Berkeley & Curtis, Linn. Soc. Bot. Jour. 10: 327. 1868; Sacc. Syll. Fung. 6: 510. 1888.

Illustrations: Lloyd, *loc. cit.*

Type: in Kew Herb. and Curtis Herb.

Fructifications resupinate, effused, gelatinous, adnate, loosening from the substratum about the margin in drying, pallid at first, now pale smoke-gray, bearing granules about 9 to the mm.; in structure 500–800  $\mu$  thick, composed of densely interwoven and crowded, suberect, gelatinous-walled, hyaline hyphae 3  $\mu$  in diameter; granules about 100  $\mu$  high, about 50  $\mu$  in diameter at the base, containing an axile sheath of fine hyphae and an accumulation of crystalline matter; basidia longitudinally septate, 15  $\times$  12  $\mu$ ; spores hyaline, even, flattened on one side, 6–7½  $\times$  4–5  $\mu$ .

Covers an area on bark of 5  $\times$  4 cm., fractured on one side and one end.

Under side of rotten logs. Cuba. January.

*Heterochaete gelatinosa* is much thicker and more gelatinous than our other American species and has smaller spores than *H. andina* and *H. sublivida*. Its fructifications are so large and thick that it should attract notice of collectors but it would probably be classed as one of the *Hydnaceae* although it must be notably gelatinous.

Specimens examined.

Cuba: *C. Wright*, 230, type (in Curtis Herb.).

*H. microspora* Burt, n. sp.

Type: in Mo. Bot. Gard. Herb. and N. Y. Bot. Gard. Herb.

Fructifications resupinate, effused, at first a white floccose mycelium which persists later as a subiculum and bears on its surface a thin, waxy, hymenial layer, pinkish buff in the herbarium, more or less cracked, and showing through the cracks the filaments of the subiculum; in structure 100–150  $\mu$  thick,



Fig 1 *H. macrospora*: Section of fructification  $\times 92$ , a, basidium, and s, spores,  $\times 665$

composed of hyaline, even, thin-walled hyphae  $2\ \mu$  in diameter, very loosely interwoven next to the substratum and with occasional crystalline masses  $6\text{--}12\ \mu$  in diameter; granules minute, numerous, protruding  $60\text{--}90\ \mu$ , containing an axile sheaf of slightly brownish hyphae and some incrusting granules; basidia longitudinally septate,  $10\text{--}15 \times 6\text{--}12\ \mu$ ; spores hyaline, even, flattened on one side,  $5\frac{1}{2} \times 3\frac{1}{2}\text{--}4\ \mu$ .

The portions of fructifications received cover areas up to  $4 \times 2\ \text{cm}$ .

On decorticated, decaying, coniferous wood. Mexico. January.

*Heterochaete microspora* is distinguished by its floccose subiculum and thin hymenial layer, small spores, and occurrence on a coniferous substratum.

Specimens examined:

Mexico: Motzorongo, near Cordoba, W. A. & E. L. Murrill, 990, type, and 995 (in Mo. Bot. Gard. Herb., 54617 and 54618 respectively, and in N. Y. Bot. Gard. Herb.).

*H. Sheari* Burt, n. comb.

*Sebacina Sheari* Burt, Mo. Bot. Gard. Ann. 2: 758. text f. 2. 1915.

Type: in Burt Herb. and in Shear Herb.

Fructifications resupinate, effused, adnate, coriaceous, with minute granules or papillae, dull white, drying pale olive-buff, cracked, the margin determinate, entire; in structure  $110\text{--}140\ \mu$  thick, with (1) a dense layer next to the substratum of longitudinally arranged, slightly brownish, even-walled hyphae  $1\frac{1}{2}\text{--}2\ \mu$  in diameter, which branch and curve outward at a right angle and form (2) a fertile less compact layer  $60\text{--}90\ \mu$  thick, of suberect, flexuous paraphyses  $3\ \mu$  in diameter, of basidia about  $15\text{--}20\ \mu$  below the surface, and of flexuous, cylindric-clavate gloecystidia  $40\text{--}45 \times 6\ \mu$ , not emergent above the surface; granules protruding  $50\text{--}150\ \mu$ , of about the same diameter at



the base, and containing an axile sheaf of brownish hyphae coming from the layer next the substratum; basidia longitudinally septate,  $15 \times 9 \mu$ ; spores hyaline, even, simple, curved,  $9-15 \times 4\frac{1}{2}-6 \mu$ .

Fructifications finally covering areas 7 cm. and more long, 1-2 cm. broad.

On dead *Berberis vulgaris* and other frondose limbs. District of Columbia and Island of Guam. October and March.

This species is noteworthy by its gloeocystidia. In the former description of this species under *Sebacina*, based on a gathering on *Berberis* in grounds U. S. Department of Agriculture, Washington, in 1902, I noted the presence of some granules on the hymenial surface. These granules are numerous in the specimen collected in 1819 on the Island of Guam, and by their structure in both gatherings require transfer of this species to *Heterochaete*. Since the only North American station is on the grounds of the United States Department of Agriculture, it seems probable that *H. Sheari* is an introduced species in our American flora coming from Guam or other distant lands of the Pacific.

Specimens examined:

District of Columbia: grounds U. S. Dept. Agr., Washington, *C. L. Shear*, 1238, type.

Island of Guam: *Edwards*, comm. by J. R. Weir, 10778 (in Mo. Bot. Gard. Herb., 56240).

#### DACRYOMYCETACEAE

Under the name *Tremella palmata*, Schweinitz described the commonest *Dacryomyces* of New England, southern Canada, and northern United States. This species ranges south to Louisiana and westward to Washington and north to Alaska. I have a single gathering on *Betula lutea* but other specimens known to me are on rotting coniferous wood. *D. palmatus* may occur as solitary or gregarious fructifications, with the lower portion tapering downward as in the authentic specimen in Schweinitz Herbarium and the illustrations by Coker and by Lloyd cited on a following page, or it may more usually and when better developed be a large, bright orange-yellow cluster of probably many fructifications so intimately coalescent as to

appear a single, many-lobed mass with no differentiated base as in the illustration in Coker's pl. 48.

*Dacryomyces palmatus* (Schw.) Burt, n. comb. Plate 3, fig. 2.

*Tremella palmata* Schweinitz, Am. Phil. Soc. Trans. N. S. 4: 186. 1832; Sacc. Syll. Fung. 6: 782. 1888; Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 151. 1920.—*Dacryopsis palmata* (Schw.) Lloyd, Myc. Writ. 6. Myc. Notes 64: 989. pl. 159. f. 1762. 1921.—*Dacryomyces chrysosperma* Berk. & Curtis, Grevillea 2: 20. 1873; Sacc. Syll. Fung. 6: 801. 1888.—*D. aurantius* Farlow, Appalachia 3: 248. 1883; Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 163. pl. 23. f. 10; pl. 48; pl. 63. f. 6, 7. 1920.—An *Dacryomyces flabellum* Ellis & Everhart, Acad. Nat. Sci. Phila. Proc. 1894: 324. 1894?

Illustrations: Coker, *loc. cit.*; Lloyd, *loc. cit.*; Gilbert, Wis. Acad. Trans. 16: 1156. pl. 83. f. 25, 26. 1910.

Type: in Herb. Schweinitz.

Fructifications gregarious or cespitose and forming erect, gelatinous, rounded, brain-like, complicated masses with surface lobed and folded, slimy when wet, cadmium-yellow to ochraceous orange and drying the same color, penetrating the bark by a whitish, radicated base; basidia forked; spores colored like the fructification, curved, becoming 5-7-septate,

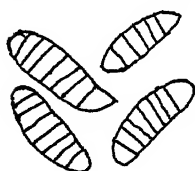


Fig. 2. *D. palm-* 18-28  $\times$  6-7 $\mu$ .

*atus*. Spores of type Mass fructifications up to 2 cm. high, 1-2 cm.  $\times$  665. broad, and 1-5 cm. long.

On coniferous stumps, logs, and brush. Canada to Louisiana and westward to British Columbia and Washington. July to March. Common in New England.

*Dacryomyces palmatus* is distinguished by its large size, bright orange-yellow color, and large 8-celled spores. Old mass forms attain the size of a large *Tremella*; some specimens of this species were distributed from Schweinitz's herbarium under the name *Tremella aurantia*. Young, gregarious specimens bear some resemblance in aspect to *Guepinia spathularia*, especially when dried, but the spores of the latter are only 8-10  $\times$  4-4½  $\mu$  and usually simple or finally becoming only 2-celled.

## Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fungi, 1697, under the name *Tremella aurantia*.

Canada: Ontario, Lake Rosseau, *E. T. & S. A. Harper*, 808; Temagami, *H. von Schrenk* (in Mo. Bot. Gard. Herb., 57049).

New Hampshire: *W. G. Farlow* (in Mo. Bot. Gard. Herb., 5304); *Miss S. Minns*, in Ell. & Ev., N. Am. Fungi, 1697; Shelburne, *W. G. Farlow* (in Mo. Bot. Gard. Herb., 57887).

Vermont: Middlebury, *E. A. Burt*, four collections: Ripton, *E. A. Burt*, two collections; Silver Lake, Salisbury, *E. A. Burt*.

Massachusetts: *Sprague*, 778, type of *D. chrysosperma* (in Curtis Herb., 6211); Worcester, *G. E. Francis*, 69.

Connecticut: Mansfield, *P. W. Graff*, 42 (in Mo. Bot. Gard. Herb., 44796).

New York: East Galway, *E. A. Burt*; Floodwood, *E. A. Burt*.

Pennsylvania: Bethlehem, *Schweinitz*, type (in Herb. Schweinitz); Carbondale, *E. A. Burt*.

South Carolina: Clemson College, *P. H. Rolfs*, 4.

Alabama: Auburn, *L. M. Underwood & F. S. Earle* (in Mo. Bot. Gard. Herb., 5299); Montgomery, *R. P. Burke*, 107 (in Mo. Bot. Gard. Herb., 21009).

Louisiana: St. Martinville, *A. B. Langlois*.

Michigan: Gogebic Co., *E. A. Bessey*, 47, 124, 154, 348 (in Mo. Bot. Gard. Herb., 56541, 56566, 56576, 56633, respectively).

Wisconsin: Dells of the Wisconsin (in Mo. Bot. Gard. Herb., 57889); Madison, *L. H. Pammel* (in Mo. Bot. Gard. Herb., 57888).

British Columbia: Vancouver Island, *W. Trelease*, 25 (in Mo. Bot. Gard. Herb., 5298).

Washington: Bingen, *W. N. Suksdorf*, 688.

*Dacryomyces abietinus* (Pers.) Schroeter, more frequently referred to as *D. stillatus*, is a common European species having spores 7-septate and of the same dimensions as those of *D. palmatus*. This species occurs occasionally in the United States; it differs from *D. palmatus* in having very small, compact fructifications which are nearly always on old, decorticated, decaying pine wood. In only one of the specimens cited below do the fructifications burst out from the bark. The name *D. stil-*

*latus* came into extensive use, because there was formerly a strong tendency among many European botanists to use the first binomial containing the true genus of the plant without regard to the priority of the specific portion of the binomial. When publishing and defining his new genus *Dacryomyces*, Nees, as he states, took Persoon's *Tremella abietina* and renamed it *Dacryomyces stillatus* Nees. How generally Nees was followed in this instance is shown in the following synonymy. It is fortunate that such cases as this are the exception. In passing it may be noted that Nees spelled his genus *Dacryomyces*.

*D. abietinus* (Pers.) Schroeter, Krypt. Fl. Schlesien 3: 400. 1888; Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 161. *pl.* 23. *f.* 12; *pl.* 63. *f.* 3, 4. 1920.

*Tremella abietina* Persoon, Obs. Myc. 1: 78. 1796; Syn. Fung. 627. 1801; Myc. Eur. 1: 104. 1822.—*Dacryomyces stillatus* Nees, System, 89. *pl.* 7. *f.* 90. 1816; Fries, Syst. Myc. 2: 230. 1823; Epier. 592. 1838; Hym. Eur. 699. 1874; Berkeley, Outl. Brit. Fung. 291. *pl.* 18. *f.* 8. 1860; Peck, N. Y. State Mus. Rept. 22: 88. 1869; Berk. & Curtis, Grevillea 2: 20. 1873; Morgan, Cincinnati Soc. Nat. Hist. Jour. 11: 94. 1888; Brefeld, Untersuch. Myk. 7: 155. *pl.* 10. *f.* 9–11. 1888; Sacc. Syll. Fung. 6: 798. 1888; Stevenson, Brit. Hym. 2: 318. 1886; Bourdot & Galzin, Soc. Myc. Fr. Bul. 25: 34. 1909.

Illustrations: Berkeley, *loc. cit.*; Brefeld, *loc. cit.*; Coker, *loc. cit.* See Sacc. Syll. Fung. 19: 536. 1910, for reference to others.

Fructifications minute, usually about 2 mm. in diameter, gregarious, sometimes touching, convex and barium-yellow at first, in drying becoming flattened, pezizoid and somewhat orange or hazel (resin-colored), attached by central part of the under side; spores colored like the fructification, curved, becoming 7-septate, perhaps rarely 9-septate,  $15-24 \times 6-9 \mu$ .

Fructifications 1–2 mm. in diameter in specimens studied by me, contracting in drying to 1 mm., sometimes longer by confluence.

• On decaying, decorticated pine and other coniferous wood. Vermont to South Carolina. Rare, but more common in Europe. August to October.

Examination of the spores should be made in case of specimens otherwise referable to *D. abietinus*, for I find by making microscopic study of specimens in published exsiccati and in herbaria that most of the specimens labeled *D. stillatus* have spores much smaller than the dimensions given above and are not more than 3-septate; such specimens are better referable to *D. deliquescens*, the species next to be considered. In the Missouri Botanical Garden Herbarium there is a specimen from Magnus under the name *D. stillatus*, and another from Berkeley in Berkeley's 'British Fungi,' No. 164, and another in Westendorp, 'Herb. Crypt.', 139; these specimens have somewhat the aspect of what they are labeled but are composed of intricately interwoven, coarse, vermiform hyphae with elongated cells containing many vacuoles and with spore-like bodies not differentiated from the hyphae; no basidia were found. These specimens are not distinguishable from the oidium stage of *D. deliquescens*, as illustrated by Tulasne<sup>1</sup> and by Falk.<sup>2</sup>

Specimens examined:

Exsiccati: Rabenhorst, Herb. Myc., 276; Ravenel, Fungi Car. 4: 81.

Sweden: Upsala, *E. A. Burt*.

Germany: in Rabenhorst, Herb. Myc., 276.

Italy: *G. Bresadola*.

Vermont: Middlebury, *E. A. Burt*.

South Carolina: Ravenel, in Ravenel, Fungi Car. 4: 81.

*D. deliquescens* (Bull.) Duby, Bot. Gall. 2: 729. 1829; Berkeley, Outl. Brit. Fung. 290. 1860; Fries, Hym. Eur. 698. 1874; Sacc. Syll. Fung. 6: 798. 1888; Morgan, Cincinnati Soc. Nat. Hist. Jour. 11: 94. 1888; Bourdot & Galzin, Soc. Myc. Fr. Bul. 25: 34. 1909.

*Tremella deliquescens* Bulliard, Herb. de la France 1: 219. pl. 455. f. 3. 1789.—*Dacryomyces minor* Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 168. pl. 49. f. left; pl. 64. f. 1-2. 1920. Illustrations: Bulliard, *loc. cit.*; Coker, *loc. cit.*; Brefeld, Untersuch. Myk. 7. pl. 9; Falk, Cohn's Beitr. Biol. Pflanzen 8. pl. 12.

<sup>1</sup>Tulasne, Ann. Sci. Nat. Bot. III. 19: 216-219. pl. 13. f. 1-3. 1853.

<sup>2</sup>Falk, Cohn's Beitr. Biol. Pflanzen 8: pl. 12. f. 3. 1902.

*f. 3.* (oidium stage). 1902; Tulasne, Ann. Sci. Nat. Bot. III. 19. *pl. 12. f. 13-19; pl. 13. f. 1-8.* (oidium stage). 1853.

\* Fructifications gregarious, small, pulvinate, avellaneo-ochraceous, somewhat wrinkled, becoming more flattened and resin-colored in drying; spores even, curved, simple, becoming 1-3-septate,  $10-14 \times 3\frac{1}{2}-5 \mu$ .

Fructifications 1-5 mm. long, 1-3 mm. broad, usually only 1-2 mm. in diameter.

Usually on decorticated, partially decayed pine and other coniferous wood but sometimes on frondose species. Vermont to Alabama, westward to Missouri, and in Alaska. March to November. Common.

*Dacryomyces deliquescens* is characterized by its small, smoky, ochraceous or pale greenish ochraceous fructifications with somewhat wrinkled surface and spores  $10-14 \mu$  long and not more than 3-septate. In most of my American gatherings on pine the fructifications are smaller than European specimens. Peck compared one of my specimens with his type of *D. minor* and reported "The spores seem too large for this. Is it not small *D. deliquescens*?"

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 333, under the name *D. stillatus*; Ravenel, Fungi Am., 135, under the name *D. stillatus*; Sydow, Myc. Germ., 555; de Thümen, Myc. Univ., 1209.

England: Epping Forest, *E. A. Burt*.

Sweden: Femsjö, *E. A. Burt*.

Germany: Brandenburg, *P. Vogel*, in Sydow, Myc. Germ., 555.

Vermont: Middlebury, 5 gatherings, *E. A. Burt*; Ripton, *E. A. Burt*.

New York: *Sartwell* (in Mo. Bot. Gard. Herb., 5301, 5302); Westport, *C. H. Peck*.

New Jersey: Newfield, *J. B. Ellis*, in Ellis, N. Am. Fungi, 333, and in de Thümen, Myc. Univ., 1209.

South Carolina: Aiken, *H. W. Ravenel*, in Ravenel, Fungi Am., 135.

Alabama: Montgomery County, *R. P. Burke*, 533 (in Mo. Bot. Gard. Herb., 57371).

Wisconsin: Madison, *W. Trelease* (in Mo. Bot. Gard. Herb., 5303).

Missouri: Meramec Highlands, *L. O. Overholts* (in Mo. Bot. Gard. Herb., 43643).

Alaska: Sitka, *W. Trelease*, 590 (in Mo. Bot. Gard. Herb., 57893); Yakutat, *W. Trelease*, 598 (in Mo. Bot. Gard. Herb., 57894).

Under the name *Tremella subochracea* Peck described a species collected by himself on decorticated wood of *Populus monilifera* at Albany, N. Y. Study of his type shows this fungus to be a *Dacryomyces* having larger and more elongated fructifications than *D. deliquescens* and slenderer spores which curve to one side below the middle into a characteristic tapering, oblique base. Spores of similar dimensions and form occur in the type of *D. minor* Pk. but in the latter the fructifications are so deeply sunk in the very rotten wood that only the upper surface is visible and I could not come to a definite conclusion in regard to the species nor the wood in which growing. A collection of mine made at Middlebury, Vt., on *Salix*, is referable to *D. subochraceus*.

*D. subochraceus* (Peck) Burt, n. comb.

*Tremella subochracea* Peck, N. Y. State Mus. Rept. 34: 43. 1881; Sacc. Syll. Fung. 6: 788. 1888.—An *Dacryomyces minor* Peck, N. Y. State Mus. Rept. 30: 49. 1879?

Type: in N. Y. State Mus. Herb.

"Small, two to four lines in diameter, forming interrupted or anastomosing lines or patches, gyrose plicate, pale-ochraceous, becoming darker in drying; spores oblong or oblong pyriform, slightly curved at the small end, colorless, .0004 in. to .0005 in. long, .00016 in. to .0002 in. broad. Decorticated wood of poplar, *Populus monilifera*. Albany. Sept. A peculiar feature of this species is its tendency to grow in lines which run together in a reticulate manner. The color is dingy-yellow or subochraceous."

Fig 3 *D. subochraceus* Basidium and spores of type × 665



The above is the original description which is of especial value in regard to the general aspect or habit of the species, for it was undoubtedly written, according to Peck's

usage, with the entire gathering of material in fresh, vegetative condition before him. In the specimens constituting the type, the fructifications while small for a *Tremella*, as published by Peck, are large for a species of the *Dacryomyces deliquescens* group, being up to 7 mm. long,  $\frac{1}{2}$ – $\frac{1}{4}$  mm. broad, now fuscous in dried condition and ochraceous drab and with surface wrinkled when softened by wetting; basidia cylindric,  $30 \times 4 \mu$ , with 2 obtuse, divergent sterigmata,  $4\frac{1}{2}$ – $6 \times 1\frac{1}{2} \mu$ ; spores continuous at first, mostly 1-septate, but becoming 3-septate,  $9$ – $13 \times 3$ – $4 \mu$ , curving below into a tapering, oblique base.

On *Populus* and *Salix*. Vermont and New York. September and November. Probably rare.

Specimens examined:

Vermont: Middlebury, E. A. Burt.

New York: Albany, C. H. Peck, type (in N. Y. State Mus. Herb.).

Still another species of the *D. deliquescens* group with large fructifications of the aspect of those of *D. subochraceus* but with broader, less curved spores was published independently by Coker and by Bresadola in 1920. This species has spores of the same dimensions and form as those of *D. deliquescens* but fructifications larger, drying paler, and occurring on frondose wood only, agreeing in these features with *D. subochraceus*. The description by Coker was published a few weeks earlier than that by Bresadola, hence the name of this species, if not too close to *D. subochraceus*, is

*D. Ellisii* Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 167. pl. 23. f. 11; pl. 50; pl. 63. f. 8. 7 Jl. 1920.

*D. Harperi* Bresadola, Ann. Myc. 18: 53. 31 Ag. 1920.

Illustrations: Coker, loc. cit.

Gregarious, bursting through the bark and forming subglobose or pulvinate, crumpled, firmly gelatinous masses, orange or wine-colored, fading to olive-buff and drying sepia and with surface plicate-gyrose, the base whitish and buried in the bark; spores hyaline under the microscope, noted by Coker as orange in spore collections,  $12 \times 5$ – $6 \mu$ .

Dried fructifications  $3$ – $5 \times 2$ – $3$  mm., and 2 mm. high.

On bark of dead limbs of alder, oak, and other frondose spe-



cies. Massachusetts to North Carolina and in Wisconsin and Illinois. October to February. Rare.

*D. Ellisii* is thicker and more pulvinate than *D. deliquescens* and has the hymenium more plicate-gyrose, broader spores, a whitish basal portion, visible upon dissecting away the outer bark, and it occurs on bark-covered limbs of frondose species; the aspect is suggestive of a *Tremella*.

Specimens examined:

Wisconsin: Madison, *W. Trelease* (in Mo. Bot. Gard. Herb., 5358).

Under the name *Dacryomyces fragiformis* (Pers.), Ellis distributed in Ell. & Ev., N. Am. Fungi, 2607, an infrequent northern species of which the specimens were collected on dead limbs of yellow birch at London, Canada, by Professor J. Dearness. *D. fragiformis* was published by Persoon as *Tremella fragiformis* and described by him as a red species occurring on dead branches of pine; in his illustration the wood is decorticated. The original description and illustration present a fungus very different from our species on birch, which is of pezizoid aspect, with yellow hymenium and white stem, and is referable to *Ditiola conformis* Karst.

*Ditiola conformis* Karsten, Notis. ur Sällsk pro Fauna et Flora Fennica Förh. 11: 223. 1871; Finska Vet.-Soc. Bidrag Natur och Folk 48: 461. 1889; Soc. Sci. Fenn. Actis 18: 110. pl. 6. f. 80. 1891; Sacc. Syll. Fung. 6: 813. 1888.

An *Guepinia Femsjoniana* Olsen in Brefeld, Untersuch. Myk. 7: 161. pl. 11. f. 3-5. 1888?

Illustrations: Karsten, *loc. cit.*

Type: Type distribution in Karsten, Fungi Fenn. Exs., 629.

Fructifications erumpent through the bark, stipitate, solitary and pezizoid or cespitose and becoming confluent and then forming pulvinate masses with hymenial surface plicate, cinnamon-buff to ochraceous buff; stem expanding above, white-floccose; basidia bifurcate; spores yellow in spore collection, simple at first, then pluriguttulate, finally 1-7-septate, 18-28  $\times$  7-9  $\mu$ .

Dried fructifications  $2 \times 2-4$  mm.; confluent masses 5-12  $\times$  5-7 mm.; stem up to 4 mm. long.

On fallen decaying branches of *Betula lutea* in mountain forests (reported by Karsten on *Alnus incana*). Ontario, Vermont, and New York. August, February, and March. Rare.

Reference of our specimens to *Ditiola conformis* has been confirmed by comparison with the type distribution by Karsten cited above; and they agree well with the description and illustration by Karsten although in America forming pulvinate masses by confluence of the hymenial portions of a cluster of fructifications. They are certainly cogeneric with *Ditiola radicata*, which I collected abundantly in Sweden but have not yet found in the United States.

Specimens examined:

Exsiccati: Karsten, Fungi Fenn. Exs., 629; Ell. & Ev., N. Am.

Fungi, 2607, under the name *Dacryomyces fragiformis*.

Canada: Ontario, London, J. Dearness, in Ell. & Ev., N. Am. Fungi, 2607.

Vermont: Ripton, Abby Pond, E. A. Burt, and Lost Pleiad Pond, E. A. Burt.

New York: Catskill Mts., C. H. Peck (in N. Y. State Mus. Herb.).

As *Tremella stipitata*, Peck described a species which has furcate basidia and spores simple at first but becoming 1-septate. The presence of a stem places this species in the genus *Dacryomitra*, as follows:

*Dacryomitra stipitata* (Peck) Burt, n. comb. Plate 3, figs. 3, 4.

*Tremella stipitata* Peck, N. Y. State Mus. Rept. 27: 100. pl. 2. f. 22, 23. 1875; Sacc. Syll. Fung. 6: 788. 1888; as *Coryne Coker*, Elisha Mitchell Scientif. Soc. Jour. 35: 150. 1920.—An *Dacryopsis ceracea* Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 175. pl. 50. f. 1; pl. 65. f. 3, 4. 1920?

Illustrations: Peck, loc. cit.

Type: in N. Y. State Mus. Herb.

"Head small, tremelloid, subglobose or irregular, glabrous,



Fig. 4. *D. stipitata*. Basidium and spores of type  $\times 665$ .

more or less uneven with gyrose convolutions, yellow, often changing to orange or reddish brown in drying; stem distinct, firm, solid, nearly equal, yellow, often tinged with brown at the base, rarely throughout its whole extent, sometimes divided at the top into two branches, each bearing a head"; basidia forked, about  $25 \times 2\frac{1}{2} \mu$ , bearing two divergent, obtuse sterigmata about  $6-9 \times 2 \mu$ ; spores hyaline, even, simple at first, becoming 1-septate,  $7-9 \times 3 \mu$ .

Fructifications 1-2 cm. high.

"On decaying wood in swamps. Forestburgh, New York. September.

"The texture of the stem is very unlike that of the head. The color of the stem generally fades to whitish or pallid in drying. The stem is sometimes slightly recurved at the top and appears to penetrate the receptacle as in the genus *Spathularia*. Barren stems occur obtusely pointed at the apex and destitute of a head."

I have the impression that I saw at one time an ample collection of the above species from New Hampshire in Farlow Herb., but I could not locate these specimens recently when desiring to make sure that their microscopic characters were like those of Peck's type. *Dacryomitra dubia* as understood by Coker appears distinct by its much larger spores. Authentic *D. dubia* Lloyd, communicated by Miss Hibbard to Lloyd, should be compared with *D. stipitata*.

A stipitate species related to the preceding was originally published as *Eridia pedunculata* B. & C. and has recently been transferred to *Dacryomyces* by Coker, but I can not reconcile the illustrations and description of his specimens with the type of *Eridia pedunculata* in its dried condition in Curtis Herbarium; it seems to me that *Dacryomyces pedunculatus* Coker is a very different species, for the original specimens of the former have slender, sulcate stems  $\frac{1}{2}$  mm. in diameter, standing up 1-2 mm. above the woody substratum and bearing at the top of each a small fertile head about 1 mm. in diameter, the general aspect of the whole fructification somewhat resembling that of a stipi-

tate Myxomycete. Since the older genus *Dacryomitra* is so broadly defined that it includes Massee's genus *Dacryopsis*—which has always been superfluous—I transfer *E. pedunculata* to *Dacryomitra*:

***D. pedunculata*** (Berk. & Curtis) Burt, n. comb.

*Exidia pedunculata* Berkeley & Curtis, Grevillea 2: 19. 1873; Sacc. Syll. Fung. 6: 773. 1888.—Not *Dacryomyces pedunculatus* Coker, Elisha Mitchell Scientif. Soc. Jour. 35: 166. pl. 23. f. 15; pl. 41. f. 4; pl. 62. f. 4, 5. 1920.

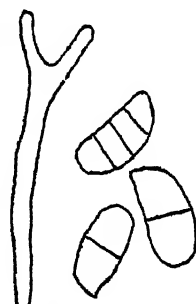
Type: in Curtis Herb. and probably in Kew Herb.

About 4 mm. high, horn color; stem erect, sulcate, bearing at the apex the expanded, lobed, and at length deflexed hymenium, about 2 mm. across; at first tuberculiform and attached by a white, floccose mycelium, which at length entirely vanishes; basidia  $40-50 \times 3 \mu$ , bearing 2 divergent, obtuse sterigmata up to  $15 \times 3 \mu$ ; spores hyaline under the microscope, thick-walled, becoming 3-septate,  $13-18 \times 6-8 \mu$ .

Dried fructifications of the Curtis Herb. specimens have heads 1 mm. in diameter, and stems 1-2 mm. long,  $\frac{1}{2}$  mm. in diameter.

On pine wood. South Carolina.

*D. pedunculata* is distinct from *D. stipitata* by much larger spores which become 3-septate. In its spore characters and occurrence on pine it agrees with *D. dubia* as understood by Coker, with dried specimens of which it should be compared.



***Dacryopsis Ellisiana*** Massee, Jour. Myc. 6: 181. pl. 7. f. 19-21. 1891; Sacc. Syll. Fung. 11: 150. 1895.—See Massee, Torr. Bot. Club Bul. 28: 519. 1901, and Durand, Torr. Bot. Club Bul. 28: 349. pl. 26, and 646. 1901.

Under the above name Massee published as a Basidiomycete an erroneous account of the structure of *Coryne Ellisii* Berk., a synonym of *Stilbium giganteum* Pk. and the imperfect stage of *Holwaya gigantea* (Pk.) Durand. The material which Massee studied was collected by Ellis at Potsdam, N. Y. I made abundant gatherings of the species on a basswood log at Middle-

bury, Vt., finding also specimens associated with the ascospore stage. In 1899, I compared my material with the type of *Dacryopsis Ellisiana* in Kew Herb., making preparations of the latter, which I still have, and studying them critically until convinced that no basidia were present and that my Middlebury gatherings agreed in all respects with the type. With regard to the final paragraph of Professor Durand's note to which reference is made above, it was published without my knowledge and I have never concurred in it. I studied the type, of which there is without doubt duplicate material in N. Y. Bot. Gard. Herb.; as a *Dacryopsis*, *Coryne Ellisii* Berk. is merely a myth of mycology.

#### AURICULARIACEAE

On a log of decayed balsa wood, *Ochroma lagopus*, received from Costa Rica, there developed in Dr. von Schrenk's rotting pit in the Missouri Botanical Garden, during April and May, 11 fructifications in various stages of development, of a tropical species of *Auricularia*, which seems undescribed, although specimens of the same species were collected in Cuba about 65 years ago and distributed by Wright under the name *Hirneola auriformis* (Schw.) Fr., from authentic specimens of which they certainly differ as noted by Farlow.<sup>1</sup>

The log on which the present gathering grew was decorticated, badly decayed, cylindric, 30 cm. in diameter by 10 cm. long, and stood erect on one end on the moist material of the rotting pit like a stump in position in the ground. Most of the first fructifications were on the least-illuminated side of the log, where they appeared at first as velvety, tubercular outgrowths 2 mm. long and 1 mm. in diameter, with obtuse ends, standing out perpendicularly from the side of the log. When 5 mm. long, the fructifications were still cylindric but curving downward at an angle of 45 degrees with the log; when 1-1½ cm. long the free end of the fructification assumed the form of a shallow cup with the concave surface facing the ground and developing an inferior hymenium, pl. 3, fig. 6. In this stage the supporting stem was attached to the center or very near

<sup>1</sup> Farlow, W. G. Bibliog. Index N. Am. Fungi 1: 305. 1905.

the center of the upper side of the pileus. In the full-grown specimens the pendant pileus expanded in a horizontal plane eccentrically to a diameter of from 6–9 cm. but with only about one-fifth of the whole diameter between the side of the log and where the stem passes into the pileus, as shown in fig. 7. Usually a short stem is present, not more than 1 cm. long, flattened, and 1 cm. in greatest diameter where it joins the pileus. The stem contracts so greatly in drying that the dried fructifications appear sessile.

In May some fructifications matured on the upper end of the log. These fructifications were cup-shaped at first, becoming expanded later, and having the hymenium superior and the stem central. In both cases, whether the pileus was pendant and with its hymenium inferior or erect and with hymenium superior, the hymenium, fig. 8, was on the surface opposite or most distant from the stem. In this connection it may be recalled that before *Hirneola* was made a synonym of *Auricularia* on account of its development the former was distinguished from the latter by a superior hymenium for *Hirneola* and an inferior one for *Auricularia*.

Stem and adjacent surface of pileus are minutely velvety with short hairs when highly magnified but to the naked eye have merely the dull texture of the petal of a rose. The color of the whole plant is somewhat shell-pink in growing specimens but became darker in drying, passing through shades of vinaceous, and finally became deep brownish drab of Ridgway, somewhat translucent, and minutely velvety. The hymenium was somewhat shining and glabrous and afforded a copious spore-fall of white spores. The flesh of the interior of the pileus was highly gelatinous, but the consistency of the whole fructification was coriaceous and pliant as rubber. For this species the following name is proposed:

*Auricularia rosea* Burt, n. sp.

Plate 3, figs. 6–8.

Type: in Mo. Bot. Gard. Herb.

Fructifications gregarious, orbicular, peltate, erect or pendant by a short stem which contracts in drying—often to a mere point of attachment—or rarely sessile from the first, soft, pliant, gelatinous within, somewhat shell-pink when grow-

ing, in drying becoming vinaceous and at length deep brownish drab, somewhat translucent, the stem and adjacent surface drying minutely velvety with hairs  $20-35 \times 3-4 \mu$ ; hymenium on the side opposite the stem, glabrous, even or with one or two shallow folds radiating from the stem; basidia flexuous, transversely septate,  $30-40 \times 4 \mu$ ; spores white in spore collection, simple, curved,  $12 \times 4 \mu$ .

Fructifications 6-9 cm. in diameter; stem, if present, up to 1 cm. long when growing.

On logs of decaying balsa wood from Costa Rica, and in Cuba.

This species may be recognized by its very thin, somewhat translucent, applanate, peltate, pendant or erect pilei of shell-pink color and texture of a rose petal when growing, and by the hymenium more even than in other species.

This species should be compared with *Auricularia lenta*, described by Fries from specimens collected at Mirador, Brazil, and known to me from only the description, with which *A. rosea* agrees in several respects.

Specimens examined:

Costa Rica: on log from there, type (in Mo. Bot. Gard. Herb., 57898).

Cuba: *C. Wright*, 286 (in Curtis Herb., under the name *Hirneola auriformis*).

There is another tropical *Auricularia* of more frequent occurrence in herbaria than the preceding species. It is

*A. delicata* (Fries) Henaings, Engler's Bot. Jahrb. 17: 492. 1893; Farlow, Bibl. Index N. Am. Fungi 1: 306. 1905; Lloyd, Myc. Writ. 5. Myc. Notes 55: 784. text f. 1177. 1918.

Plate 3, fig. 5.

*Laschia delicata* Fries, Linnaea 5: 533. 1830; Epicr. 499. 1838; R. Soc. Sci. Upsal. Acta III. 1: 105. 1851; Sacc. Syll. Fung. 6: 407. 1888.—*L. tremellosa* Fries, Summa Veg. Scand. 325 (foot note). 1849; R. Soc. Sci. Upsal. Acta III. 1: 105 (as synonym). 1851; Sacc. Syll. Fung. 6: 407. 1888.—*Auricularia tremellosa* (Fries) Patouillard, Jour. de Bot. 1: 226. pl. 4. f. 9, 10. 1887; Farlow, Bibl. Index N. Am. Fungi 1: 309. 1905.

Illustrations: Lloyd, loc. cit.; Patouillard, loc. cit.

Somewhat orbicular or shell-shaped, sessile and attached by

the margin or marginate all around and pendant by a short stem attached to the upper side near the margin, drying very thin, somewhat translucent, buffy brown to fuscous, with upper surface more or less minutely velvety and somewhat veined; hymenium inferior, forming rather deep, angular pores about 1-2 mm. in diameter and about half as deep in the dried herbarium specimens, with the more prominent walls somewhat radiating from the stem; basidia flexuous, transversely septate,  $30-45 \times 4\frac{1}{2}-5\frac{1}{2} \mu$ ; spores hyaline, even, simple, curved,  $9-12 \times 4-5\frac{1}{2} \mu$ .

Dried fructifications 2-4 cm. in diameter and  $\frac{1}{2}$  mm. thick.

On dead wood. West Indies and Mexico. December to April.

This species is distinguished by having its hymenium in irregular folds and pits, as in *Merulius tremellosus*, to so marked a degree that dried specimens are likely to be regarded as a dark species of *Merulius*, from which the slender, transversely septate basidia readily separate it.

Specimens examined:

Exsiccati: Smith, Central American Fungi, 142.

Cuba: *C. Wright* (in Curtis Herb.).

Jamaica: Balaklava, *A. E. Wight*, 306, 309, and 342 (in Farlow Herb.).

Mexico: Jalapa, *C. L. Smith*, in Smith, Cent. Am. Fungi, 142; Motzorongo, *J. G. Smith* (in Mo. Bot. Gard. Herb., 480); Orizaba, *J. G. Smith* (in Mo. Bot. Gard. Herb., 4066).

There occurs throughout North America on prostrate, decaying trunks and limbs of *Populus tremuloides* a common and conspicuous species which I have determined during many years for my correspondents as *Phlebia strigoso-zonata* (Schw.), for I had compared my collection with the type of *Merulius strigoso-zonatus* Schw. in Herb. Schweinitz. The combination *Phlebia strigoso-zonata*, with the alternative *Auricularia strigoso-zonata* (Schw.) Lloyd under his pseudonym McGinty, was finally published by Lloyd, Myc. Writ. 4: Letter 46: 6. 1913, and regarded as synonymous with a species of the Far East known as *Auricularia rugosissima* (Lév.) Bres., as well as by other names.

*Auricularia rugosissima* is known to me by the specimen from



the Philippine Islands distributed in Sydow, Fungi Exot. Exs. 321, as well as by two other Philippine collections, viz., that from E. D. Merrill, 3508, and the other by H. M. Curran, Forestry Bureau, 8907.

There is a close resemblance in aspect and coloration between the above-mentioned specimens of *A. rugosissima* and our American *Phlebia strigoso-zonata*, but the latter has simple basidia bearing 4 spores at the apex on slender sterigmata. The demonstration of these basidia is easy, for in a fertile specimen the mature basidia protrude beyond the dense, compact, dark layer of hymenial hairs and stand out conspicuously, bearing their spores. One should disregard the difficult structure of this dark layer and run along its edge in the section for the more or less scattered exserted basidia. My demonstration has been confirmed many times by members of my classes in mycology who have used fertile specimens of this species in laboratory work in determination of genera.

Hence *Merulius strigoso-zonata* Schw. is not a species of *Auricularia* but should be included in *Phlebia* on account of the configuration of its hymenium and simple basidia. The present status of this species so far as known to me from examination of authentic specimens is as follows:

*Phlebia strigoso-zonata* (Schw.) Lloyd, Myc. Writ. 4. Letter 46:6. 1913; Kauffman, N. Y. State Mus. Bul. 179: 88. 1915.

*Merulius strigoso-zonatus* Schweinitz, Am. Phil. Soc. Trans. N. S. 4: 160. 1832.—*Auricularia strigoso-zonata* (Schw.) Lloyd, Myc. Writ. 4. Letter 46:6. 1913.—*Phlebia rubiginosa* Berkeley & Ravenel in Ravenel, Fungi Car. 3: 23. 1855; Grevillea 1: 146. 1873; Sacc. Syll. Fung. 6: 499 —*P. pileata* Peck, N. Y. State Mus. Rept. 29: 45. 1878; Sacc. Syll. Fung. 6: 499. 1888. —An *Phlebia orbicularis* Berkeley & Curtis, Hooker's Jour. Bot. 1: 237. 1849, and Grevillea 1: 146. 1873?

Type: in Herb. Schweinitz.

Fructifications coriaceous, resupinate or effuso-reflexed, with the pilei more or less imbricated and laterally confluent, concentrically sulcate, zonate, somewhat tomentose, drying Natal-brown or Hay's brown, with usually 1-3 narrow, darker, alternating zones; hymenium becoming crowded with slightly elevated,

radiating folds or wrinkles which are frequently interrupted, drying fuscous to fuscous-black and finally suffused with a bloom, the margin red or orange when young; basidia simple, hyaline, protruding beyond the dark zone of hymenial hairs,  $12-15 \times 3-4 \mu$ , bearing 4 slender sterigmata  $4\frac{1}{2} \mu$  long; spores white in collection on slide, flattened on one side,  $6-8 \times 3-4 \mu$ .

Resupinate fructifications 1-5 cm. in diameter; reflexed fructifications have reflexed part up to 5-15 mm. long and resupinate portion up to 10 cm. in diameter.

Common on poplar, reported on beech and oak also. Ontario to South Carolina and westward to Manitoba, Minnesota, and Arkansas. August to December.

Early in its season the fructifications of this species are small, resupinate, brighter red than later, and with the hymenium nearly even and not yet fertile. The type specimen of *P. orbicularis* has the aspect of an immature specimen of *P. strigosozonata*, but it may prove distinct since it was collected on *Quercus*; in its native region, it should be followed through the season until it gives good spore collections in order that mature specimens may be available for comparison with *P. strigosozonata*. Specimens referable to the latter were distributed under various names in Ell. & Ev., N. Am. Fungi, 2731, and 3416, the latter being fertile, in Ravenel, Fungi Car. 3: 23, and in Shear, N. Y. Fungi, 47.

***Helicobasidium Peckii* Burt, n. sp.**

Type: in Mo. Bot. Gard. Herb. and N. Y. State Mus. Herb.

Fructification resupinate, effused, coriaceous, separable, drying with the subiculum loosely interwoven and army-brown and the hymenium avellaneous, even, dry, glabrous, not at all gelatinous



Fig. 6. *H. Peckii*. Young basidium, *b'*, mature basidia, *b*, and spores, *s*, type,  $\times 665$ .

or waxy; in structure 800-1200  $\mu$  thick, composed of loosely interwoven, stiff, colored hyphae  $4\frac{1}{2} \mu$  in diameter, not nodose-septate, not incrustated, darkest next to the substratum; basidia curved or hook-shaped, becoming transversely 3-septate, with the sterigmata distributed one to each cell on the convex side; spores hyaline, even, flattened on one side,  $9 \times 6 \mu$ , copious.

Fructification 4 cm. in diameter.

On spruce bark. Adirondack Mts, N. Y. June 7, 1905.  
Probably rare.

The general appearance of *H. Peckii* is that of a *Corbicium*, *Coniophora*, or *Hypochnus*, with the coffee-colored hymenium covering the reddish brown subiculum. The basidia are not crowded together as closely as in most species of *Corbicium* and show well their hook-shaped form when thin sections are examined. *Helicobasidium* is a small genus and has not been recorded heretofore for America. I am indebted for the privilege of studying the present specimen to Dr. H. D. House who found it among the undetermined collections of Peck, to whose memory I dedicate the species in grateful regard for assistance and friendship which began in 1879.

## EXPLANATION OF PLATE

### PLATE 3

All figures natural size

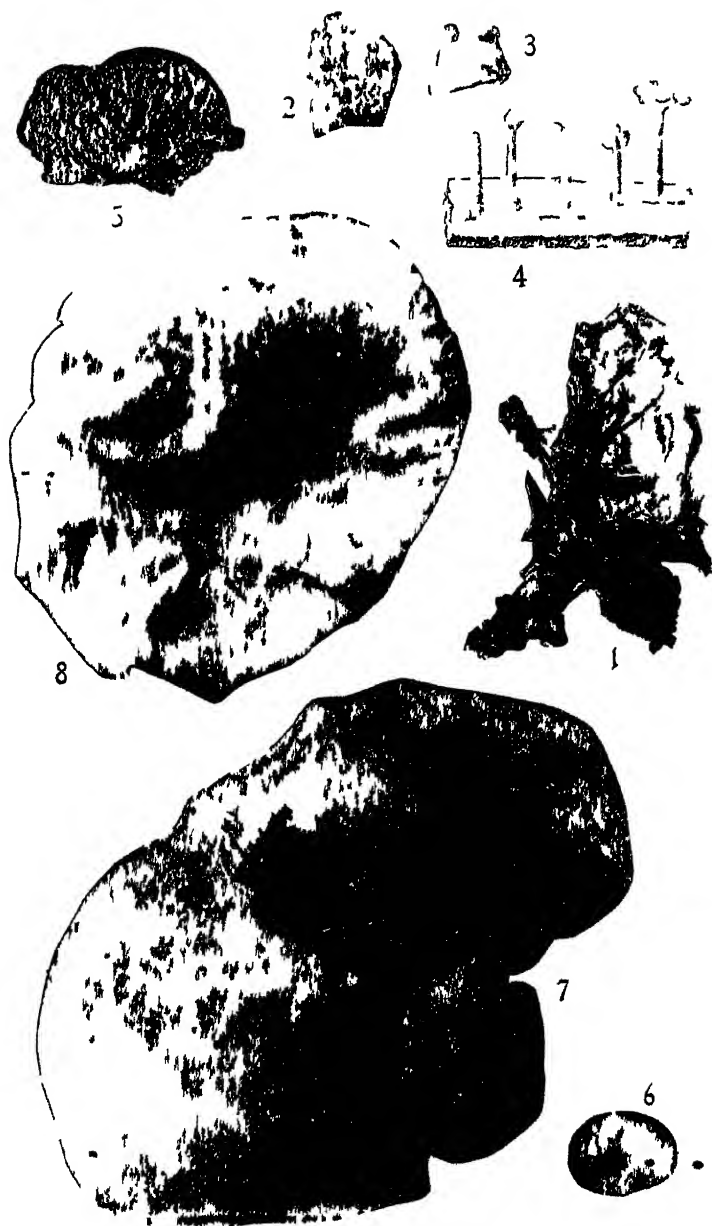
Fig 1 *Tremella concrescens* From type of *Dacryomyces pellucidus* in Herb Schweinitz

Fig 2 *Dacryomyces palmatus* From type of *Tremella palmata* in Herb Schweinitz.

Figs 3, 4 *Dacryomitra stipitata* Fig 3, from the type in N Y State Mus Herb, Fig 4, after the illustration in N Y State Mus Rept 27 pl 2 f 22

Fig 5 *Auricularia delicata* Collected at Motzorongo, Mexico, by J G Smith, in Mo Bot Gard Herb

Figs 6-8 *Auricularia rosea* From the type in Mo Bot Gard Herb, Fig 6, small fructification in fresh condition, Figs 7 and 8, mature fructification in vegetative condition, 7, showing stem and adjacent surface and, 8, hymenial surface





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